

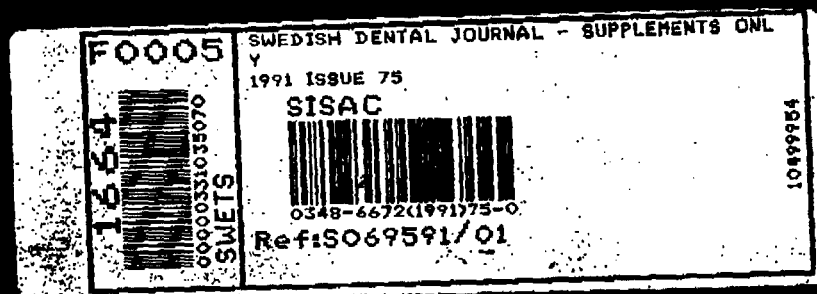
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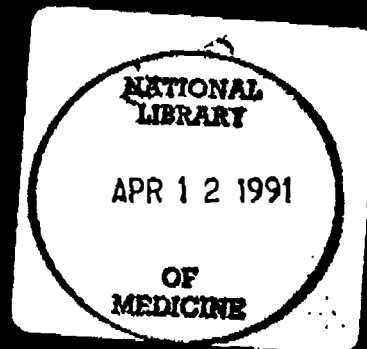
**SNUFF-INDUCED CHANGES
ASSOCIATED WITH THE USE OF
LOOSE AND PORTION-BAG-PACKED
SWEDISH MOIST SNUFF**

**A clinical, histological and
follow-up study**

Gunilla Andersson



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to
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PREFACE

The present thesis is based on the following papers which will be referred to by their Roman numerals

- I. Andersson G, Axéll T. Clinical appearance of lesions associated with the use of loose can packed and portion-bag packed Swedish moist snuff - a comparative study. J Oral Pathol Med 1989; 18: 2-7.
- II. Andersson G, Axéll T, Larsson Å. Histologic changes associated with use of loose and portion-bag packed Swedish moist snuff - a comparative study. J Oral Pathol Med 1989; 18: 491-7.
- III. Andersson G, Axéll T, Larsson Å. Impact of cosumption factors on soft tissue changes in Swedish moist snuff users - a histologic study. J Oral Pathol Med 1990; 19: 453-8.
- IV. Larsson Å, Axéll T, Andersson G. Reversibility of snuff dipper's lesion in Swedish moist snuff users - a clinical and histologic follow-up study. J Oral Pathol Med 1991; 21 (*in press*).
- V. Andersson G, Axéll T, Larsson Å. Clinical classification of snuff dipper's lesions supported by histology. J Oral Pathol Med 1991; 21 (*in press*).

INTRODUCTION

Snuff manufacturing in Sweden started in the early eighteenth century. During the following centuries the use of snuff increased in Sweden. At first, snuff was used as a fine, dry powder that was inhaled through the nose but from the end of the nineteenth century moist snuff has been used intraorally as a quid, most commonly placed inside the upper lip in the vestibular area. From the early 1900s more snuff per capita was sold in Sweden than anywhere else in the world. In 1919, 7,000 tons (1.5 kg per capita) of moist snuff were sold, but after 1920 the consumption gradually decreased until 1969. During the last two decades snuff sales have again increased and in 1989 4,850 tons were sold (0.6 kg per capita). About 15 years ago a new smokeless tobacco product, portion-bag-packed snuff, was introduced on the Swedish market. The sales have increased steadily and comprised about 10% (480 tons) of the total amount of smokeless tobacco sold in 1989. In the same year, only 14.5 tons of chewing tobacco was sold.

In a report from the International Agency for Research on Cancer*, it was stated that "there is sufficient evidence that oral use of snuff of the types commonly used in North America and western Europe is carcinogenic to humans". This conclusion was supported by the U.S. Surgeon General in a Surgeon General's report in 1986. This categorical statement is, however, contradictory to epidemiological data from Sweden, where the incidence of intraoral cancer is comparatively low (Waterhouse et al 1976). Further, the number of cancer cases that might possibly be associated with the use of snuff is restricted to a very few (Axéll, Mörnstad & Sundström 1978) in spite of the large number of snuff-users (about 800,000**).

Partly because of these contradictory opinions, it is important to analyse the nature of snuff-induced changes in Sweden. Almost every Swedish snuff-taker exhibits a change of the oral mucosa at the site(s) where tobacco is regularly held, "snuff dipper's lesion", which should not be considered equivalent to the precancerous lesion of leukoplakia (Axéll 1976, Axéll 1987).

* IARC MONOGRAPHS on the evaluation of the cancer risk of chemicals to humans, vol 37, 1985

** Annual report from the Swedish Tobacco Company on snuff-users in Sweden.

An analysis of the nature of snuff-induced oral mucosal changes should preferably be based on follow-up studies including clinical as well as histological examinations of such changes. This analysis should make it considerably easier to suggest recommendations for surveillance of subjects with a regular snuff habit.

HISTORICAL REMARKS

An extensive account of the history of smokeless tobacco is given in review articles by Stewart 1967 and Christen et al. 1982. A few remarks are given below.

South-American Indians were the first people known to use snuff, and they did so as early as in the fifteenth century. Tobacco leaves were pulverised in a cup of rosewood and the powder was placed in bone tubes. Snuff was brought to Europe by a Franciscan monk, Friar Ramón Pané. He travelled with Christoffer Columbus on his second voyage to the New World in 1493 and met the Carib Indians, who used snuff. In Haiti, snuff powder was used to clear nasal passages and as an analgesic by medicine men. During the sixteenth century tobacco powder was also used by Mexican Indians to heal burns and wounds, to send them to sleep and to reduce pain. The Indians inhaled powdered tobacco through a hollow Y-shaped piece of cane or pipe by placing the forked ends into each nostril and the other end near the powdered tobacco. This instrument was called a "tobago" or "tobaca". In the same century, the Spaniards started to grow tobacco and they later on changed the name of the instrument to tobacco. The French ambassador to Spain in the mid sixteenth century, Jean Nicot, who was also an amateur botanist, brought tobacco to France. He introduced it as a medical drug by giving it to the Queen of France, Catherine de Medici, to cure her son's headaches. The powdered tobacco was named snuff by the Dutch in the mid sixteenth century. Conrad Gessner, botanist, physician and scientist in Zürich, referred to the plant as *Nicotiana* in latin, in honour of Jean Nicot.

In 1753 the Swedish biologist Carl von Linné published *Species Plantarum*. The modern scientific terms of the genus *Nicotiana*, *tabacum* and *rustica*, were first included here, and tobacco was thereby definitely classified as a member of the *Solanaceae* family.

In the mid seventeenth century snuff use had spread throughout South America, China, Japan, Africa and also within Europa to England, Scotland, Ireland and Sweden. At this time snuff was used as a medical remedy but in some countries snuff use was also considered to be an "aristocratic" habit.

The first time snuff is mentioned in Swedish history is in 1637 in letters written by Axel Oxenstierna (W Løwne, personal communication). That year, 1 pound (sic!) of "snuff tobacco" was brought into Finland and registered in customs statistics from Borgå. Snuff was made really popular in Sweden in the eighteenth century by King Gustav III. Use of nasal snuff was highly fashionable at the court but when it gradually spread to the working class a moist variant was preferred. Since the beginning of eighteenth century snuff has been manufactured in Sweden. Henrik Stegman, who had a tobacco spinning mill in Stockholm in the early eighteenth century, was probably the first producer of snuff in Sweden. In about 1770 Petter Swartz started large-scale production of snuff in Norrköping. In 1795 Samuel Fielder established a snuff mill in Gothenburg and started a small business, which later developed into three separate companies. At the end of the nineteenth century the leading company was situated in Stockholm. Jacob Ljunglöf was then the leading producer and introduced the brand "Ettan" in Sweden and all over Europe. In 1914 the Swedish tobacco monopoly took over the production of snuff. Around 1920 a large factory was built in Gothenburg, making this city the centre of snuff production in Sweden.

PRODUCTS AND PRACTICES

~~The term smokeless tobacco is used to describe any of a variety of products~~ and mixtures that contain tobacco as the principal constituent and which are consumed without combustion. Although there is ethnographic evidence of tobacco ingestion in almost any conceivable way, oral and nasal consumption are the only ways practised today in the industrialised world.

Smokeless tobacco products may be subdivided into snuff and chewing tobacco. A classification of smokeless tobacco is shown in Figure 1 in Paper I. This classification is based on the physical state of the tobacco in the finished product (whether the tobacco has been pulverised or not) rather than on the way it is used and it is in accordance with American and Swedish linguistic usage. It should be pointed out, however, that in, for example, British English, the word "snuff" is commonly associated with nasal and the word "chewing tobacco" with oral use. These linguistic differences sometimes lead to confusion in the interpretation of the literature on smokeless tobacco.

Snuff-taking

Snuff is a term used for a wide variety of smokeless tobacco products comprised of tobacco which has been ground or cut into fine particles. In Europe and the USA, snuff is available as products with different particle sizes and moisture contents and with or without added flavours. The two main types are moist snuff and dry snuff. Either type may or may not have been subjected to a fermentation process during manufacture. The fermentation is a spontaneously occurring biochemical process in the moistened tobacco which causes profound chemical changes in the tobacco. Moist snuff is used exclusively in the mouth. The water content varies according to brand between 35 and 60 per cent. Dry snuff, in which the tobacco is processed into dry powdered flour, can be placed in the oral cavity or used nasally. It has a moisture content of less than 15%. In the USA a special type of fermented dry snuff ("Scotch type snuff") is used mostly by middle-aged and older people, especially in the rural South, while young adults mainly use fermented moist snuff.

Swedish "snus" is a non-fermented variety of moist snuff. Instead of being fermented, the ground tobacco, after addition of salt and water, is subjected to a heat treatment process which renders it practically free from microorganisms, lowering the risk of nitrate formation and subsequent formation of nitrosamines. Sodium carbonate is added to raise the pH of the "snus" to 8-9 in order to facilitate nicotine absorption through the oral mucous membranes. The nicotine content of the various brands is in the order of 5 to 11 mg/g of the moist product. The most common type in Sweden is loose moist snuff used as a 1-2 g quid formed with the fingers and placed in the mouth. The other type, portion-bag-packed snuff, consists of an 0.5 or 1 g portion of moist snuff packed in small sachets made of nonwoven paper-like material similar to that used for tea bags. In Sweden, the overwhelming majority of snuff-users place the quid in the vestibular area inside the upper lip. About twenty different brands of snuff, most of them flavoured, are available on the Swedish market.

Snuff habits in Sweden - demographic data

In 1955 (SCB* us) snuff use was most common among pensioners while no users were registered below the age of 20. The proportion of snuff-users among men has increased from 14% in 1977 to 19% in 1986 (NTS**/ SIFO*** 1977-83, NTS/SCB 1984-86). This increase is mostly due to the fact that daily snuff use is today also common among younger men; 35% of men aged 16-24 years and 26% of men in the agegroup 25-34 years use snuff every day. Snuff use is more prevalent in sparsely populated area than in densely built-up areas, although this difference has decreased among men aged 18-34 years as compared to men aged 55-70 years. Daily snuff use is more common in the northern part of Sweden and among men with no or a short education compared to those with at least 3 years in a continuation school. Among Swedish women, only about 1% are daily snuff-users.

* National Central Bureau of Statistics

** The National Smoking and Health Association

*** The Swedish Institute of Public Opinion Research

Tobacco-chewing

Chewing tobacco is a non-combustible, heavily cased tobacco product. The main types of chewing tobacco are plug, twist or roll and looseleaf. The twist type is often cut into pieces of suitable portion size. The predominant raw material for chewing tobacco is heavily-bodied, fire-cured tobacco. The difference in taste between the various brands on the market lies mostly in the casing, which may be sweet, sharp, salty or fruity. Some chewing tobaccos have been fermented during manufacture.

The habit of tobacco-chewing is practised mainly in the USA and South East Asia and to some extent in Europe (Scandinavia, Bavaria, Wales).

Examples of other smokeless tobacco habits

In South-East Asia and Africa, different kinds of snuff are used orally and nasally. For instance, in Thailand a tan and dry powder is inhaled through the nose. In South Africa, tobacco leaves mixed with a lot of additives (aloe, oil, juice, herbs) are used nasally but in some areas the product is placed in the mouth. In Sudan, the snuff is named *Saffa*. It is a moistened powdered tobacco treated with soda, used orally and placed mostly behind the upper lip. In India, unscented snuff is used as a dentifrice, while scented snuff is used for sniffing. In parts of the Orient, especially in India, and among East Indians in Africa, the chewing of betel quid is widespread. A betel quid may be composed of betel leaf, areca nut and lime and is used with or without the addition of crushed tobacco leaves.

In India, powdered tobacco with lime, named *Khaini*, is used mostly as snuff and placed in the mouth, generally in one or both cheeks. *Mishri* is used as a substitute for chewing tobacco primarily for cleaning the teeth but the use frequently becomes habitual. *Zarda* is comprised of tobacco leaves with addition of lime and spices and is usually chewed mixed with areca-nuts. It is produced and used in India but also exported to a number of Arab countries. In some parts of southern Saudi Arabia, *Shammah* is used. It is the native name for a mixture of powdered tobacco leaf, carbonate of lime or soda and other substances including ash. The quid is placed in the buccal or lower labial vestibular area of the mouth.

In Iran and the Soviet central Asian republics, a special product, *Nass*, is used. It is made of regional tobacco and mixed with ash, cotton oil and lime. There are regional differences in the composition as well as in the anatomical site in the mouth where it is placed.

In Afghanistan and Pakistan, *Naswar* is used. It is a mixture of powdered tobacco, slaked lime and indigo. Mostly it is kept in the floor of the mouth.

GENERAL BACKGROUND

Epidemiology of oral snuff-induced lesions in Sweden

In 1976 Axéll published data on 20,333 Swedish people examined in 1972-73, living in the central part of Sweden, aged 15 years or above. The frequency of participation was 89.7% of the total adult population in the investigated area. The prevalence of snuff-dipping was 15.9% among men and 0.2% among women, comprising 8% of the total population. Almost all of them showed some degree of snuff-dipper's lesion.

Salonen, Axéll & Helldén (1990) examined a randomly selected sample of 918 (corresponding to about 95% participation) adult subjects, 448 men and 470 women, living in a Swedish county, for the presence of oral mucosal lesions associated with snuff use. The diagnostic criteria were largely the same as used by Axéll in 1976. Seventynine (17.6%) subjects, all males, were snuff-users, corresponding to 8.6% of the total population. Snuff dipper's lesion was recorded in 63 cases, comprising a total prevalence of 7.2%.

Other population data from Scandinavia

Roed-Petersen & Pindborg (1973) examined 450 Danish men (mean age 58.4 yrs) with oral leukoplakias. Thirty-two (7%) patients were snuff-dippers and showed a mucosal lesion which was called snuff-induced oral leukoplakia. Patients with leukoplakia due to snuff use experienced fewer symptoms than did patients with leukoplakia due to other, unknown causes. They also tended to be older at the first examination and to have a higher alcohol intake.

Jungell & Malmström 1985 reported on 441 Finnish recruits aged 17-29 years. Forty-eight (11%) snuff-users were identified, out of whom 36 showed snuff-induced lesions. Among those who did not show any changes of the mucosa, 8 had given up the habit three weeks before examination and 4 had used snuff for only 2-3 weeks.

Clinical picture of snuff-induced changes

Oral mucosal changes associated with the use of snuff have been the subject of many studies. They have been described as showing a white or yellowish to brownish, wrinkled or folded, surface, often with a pattern of delicate white striae. The changes can show varying degrees of thickness and diffuse blending into the surrounding normal mucosa (Pindborg & Renstrup 1963, van Wyk 1965, Archard & Tarpley 1972, Roed-Petersen & Pindborg 1973, Axéll, Mörnstad & Sundström 1976, Frithiof, Anneroth, Lasson et al. 1983, Hirsch, Heyden & Thilander 1982, Greer & Poulson 1983, Poulson, Lindemuth & Greer 1984, Jungell & Malmström 1985, Grady, Greene, Daniels et al. 1990). The changes are recorded on the site(s) where the snuff quid is regularly placed. They have been labelled snuff dipper's lesion (Axéll, Mörnstad & Sundström 1976, Smith, Mincer, Hopkins et al. 1970, Mörnstad, Axéll & Sundström 1989) but also snuff-induced oral leukoplakia (Roed-Petersen & Pindborg 1973), snuff-induced lesion (Hirsch, Heyden & Thilander 1982, Frithiof, Anneroth, Lasson et al. 1983, Jungell & Malmström 1985) and snuff dipper's keratosis (Archard & Tarpley 1972).

Axéll, Mörnstad & Sundström (1976) introduced a four-point scale for classification of snuff dipper's lesion and gave clinical criteria. The results from that study indicated that subjects with a regular snuff habit may develop a more pronounced lesion with increasing consumption. These results were partly supported by a study by Hirsch, Heyden & Thilander (1982) showing that patients with changes of the fourth clinical degree had been snuff-dippers for significantly more years than patients showing changes of the other three degrees. There were also considerable differences between the clinical grading groups concerning daily consumption of snuff. Mörnstad Axéll & Sundström (1989) evaluated the impact of consumption parameters on the clinical lesion in 1,459 Swedish male snuff-dippers. The severity of the lesion was more pronounced after longer periods with the habit, with the exception of the group with the longest period, 60 - 80 years, of snuff use. There was also a positive correlation between daily consumption of snuff (t/day and g/day) and the severity of lesions but only to some extent with the age of the snuff-dipper. There was a slight tendency to less severe lesions if the quid was placed at more than one site.

Greer & Poulson (1983) published a study on oral tissue alterations associated with the use of smokeless tobacco by 1,119 teenagers; 117 (10.45%) of them were snuff-users. Since they could not find any degree 4 lesion, the classification by Axéll was modified to a three-point scale described largely by the same criteria as for degrees 1-3 in the four-point scale. The average exposure time (yrs and h per day) for those with clinical lesions was higher than for those without oral signs. However, the subjects of this study had a lower daily consumption for rather few years compared to the adult snuff-dippers in the Scandinavian studies. In a comparative study (Poulson, Lindemuth & Greer 1984) of the use of smokeless tobacco in 56 subjects out of 445 rural and urban teenagers, it was further demonstrated that those who exhibited oral sequelae reported greater daily exposure and a longer history of use than users without oral sequelae. It was pointed out, however, that even if the development of oral sequelae is dose-related on average, individual variability or susceptibility does exist. At first, Greer & Poulson (1983) presumed that the three-point scale should be applied to persons who have used smokeless tobacco for a comparatively short period of time (4 yrs or less). All the same, in a study on smokeless-tobacco-associated oral changes in juvenile, adult and geriatric patients (Greer, Poulson, Boone et al. 1986) it was possible to classify all lesions on the three-point scale in spite of the fact that the exposure time in this population ranged from 8 months to 36 years. No data on daily exposure were given in this study, however.

Wolfe & Carlos (1987) studied 226 Navajo Indians, aged 14-19 years. Smokeless tobacco was used by 64.2% of the subjects and 25.5% had leukoplakia, classified on a three-point scale. In this study, the duration (years) and the frequency (days per week) of use were the only significant determinants of the presence, but not the severity, of leukoplakia in users. Further, the prevalence of leukoplakia only increased with increasing exposure time in years up to 4 years.

Grady, Greene, Daniels et al. (1990) examined 1109 members of baseball teams. A four-point scale was used to classify lesions recorded as leukoplakia/erythroplakia. The number of hours tobacco was held in the mouth, hours since last use, type (snuff versus chewing tobacco) and brand of snuff were significantly associated with risk for developing leukoplakic lesions among smokeless tobacco users. However, the only independent

predictor of the severity of the lesion was the amount of smokeless tobacco used daily, recorded as number of hours of oral mucosal snuff exposure.

Gingival recessions

When examining 50 snuff-users in South Africa, van Wyk (1965) noted that the necks of teeth adjacent to the clinical mucosal lesions recorded were abnormally exposed and stained black. He suggested that the gingival recessions were caused by chronic inflammation of the gingiva brought on by the injurious properties of snuff.

Modeér, Lavstedt & Åhlund (1980) published a study on 232 schoolchildren in Sweden. Among the boys 11% were regular snuff users. Snuff use showed a significant correlation to gingivitis after controlling for plaque. They concluded that snuff-taking may influence the gingival tissue directly and cause gingivitis.

Greer & Poulson (1983) defined tobacco-associated gingival recessions as apical migration of the gingiva to or beyond the cemento-enamel junction, with or without clinical evidence of inflammation. Out of the 117 smokeless tobacco users, 57(48.7%) subjects had soft tissue lesions. Fifty(42.7%) had oral mucosal lesions and 23(19.6%) of these had gingival recessions as well and 7(6%) had gingival lesions alone. However, they concluded that no advanced tobacco-associated periodontal deteriorations were demonstrated, which might be explained by the fact that no long-term users took part in their study. These results were confirmed in a study by Poulson, Lindemuth & Greer (1984). They used the same criteria when examining 56 (12.6%) snuff-users out of a total sample of 445 teenagers. Soft tissue lesions were recorded in 35 (62.5%) boys. Thirty-three (58.9%) showed oral mucosal lesions; 13 (23.2%) of these had gingival recessions as well and 2(3.5%) had gingival lesions alone.

Frithiof, Anneroth, Lasson et al. (1983) studied 21 male snuff-users. All of them had oral mucosal lesions. Gingival retraction was observed in 2 cases, defined as an affected gingiva which was whiter than normal but also including reddish areas.

Offenbacher & Weathers (1985) studied the effect of smokeless tobacco on the periodontal and mucosal status of 565 males with a mean age of 13.8 years. Altogether 75(13%) used smokeless tobacco. Gingival recessions were recorded in 45(60%) of them and mucosal pathology in 17(23%) subjects. Their results are somewhat confusing since they reported that there was no relationship between the use of smokeless tobacco and the incidence of gingivitis. On the other hand, they concluded that smokeless tobacco use was a significant risk factor for development of gingival recessions but only in individuals with co-existing gingivitis. No data were given on the number of smokeless tobacco users with combination of these two oral conditions.

Wolfe & Carlos (1987) examined 226 teenagers. They did not find any differences in prevalence of gingival recessions between snuff-users and non-users. Neither were there any recession differences between quid placement segments and control segments within the same individual.

Histological features

The first publication with comments on the histopathology of the epithelium in snuff-induced oral leukoplakias is an article by Pindborg & Renstrup published in 1963. It reported on 12 men, who were regular users of "Göteborg snus" for 20-50 years. Histologically, a marked hyperplasia of the outer epithelial layers with large and vacuolated cells was found. The epithelial surface was unkeratinised. A few streaks of parakeratosis were found deeper in the epithelium.

Van Wyk (1965) reported on oral lesions caused by snuff. He examined 50 snuff lesions, 25 histologically and 25 cytologically. The duration of these lesions ranged from a few weeks to 40 years. In the histological analysis, he found hyperplastic, acanthotic and parakeratotic epithelial layers overlying a chronically inflamed lamina propria. In 4 cases he recorded a "disquiet epithelium". These lesions showed numerous mitotic figures and disruption or fragmentation of the basement membranes. Associated changes such as pleomorphism and hyperchromatism were also present. None of these 4 subjects was either the longest or the heaviest snuff taker. Van Wyk concluded that the clinical impression was that no correlation exists between oral carcinoma and oral snuff-taking, but the histological findings indicated

that much more intensive study of the effects of snuff on living tissue is advisable.

Smith, Mincer, Hopkins et al. (1970) examined 15,000 snuff users. In only 1,751 patients did he find oral mucous membrane changes. One hundred fifty seven of these showed tissue changes that they thought should be biopsied. However, none of the biopsies showed changes consistent with dyskeratosis or malignancy. A follow-up study was carried out 4.5 years later. Of the initially examined patients, 201 were lost to follow-up. Among the remaining 1,550, 128 underwent re-biopsies. The findings from the initial histological analyses were confirmed.

The histological features characteristic of snuff induced lesions were thoroughly analysed and described in a study by Roed-Petersen & Pindborg (1973). Their findings have been confirmed in studies from, above all, Scandinavia and the USA with addition of other observations. (Axéll Mörnstad & Sundström 1976, Pindborg, Reibel, Roed-Petersen et al. 1980, Hirsch, Heyden & Thilander 1982, Frithiof, Anneroth, Lasso et al. 1983, Greer & Poulson 1983, Poulson, Lindemuth & Greer 1984, Jungell & Malmström 1985, Greer, Poulson, Boone et al. 1986). The histopathology of the snuff-induced lesion was described as consisting of hyperplastic epithelium (87.1%) with keratinisation, predominantly hyperparakeratosis, which in half of the cases was composed of vacuolated cells with streaks of parakeratinised cells in between, later on called the chevron type of keratinisation. Inflammation of varying degrees was recorded. In 8 out of 13 biopsies in which salivary glands were present, hyalinised and eosinophilic deposits were demonstrated.

In a study on tobacco-induced changes in oral leukoplakic epithelium, Pindborg, Reibel, Roed-Petersen et al. (1980) concluded that the chevron type of keratinisation was only found in tobacco-users. The snuff-induced changes appeared to have a higher number of vacuolated cells between the keratinised peaks than changes associated with other types of tobacco use. Clinically, this particular keratinisation pattern was often characterised by a pumice-like appearance.

In addition to the histological features described above, Axéll, Mörnstad & Sundström (1976), in 114 biopsies from snuff dipper's lesions, found

eosinophilic granulocytes in the epithelium in a few cases. No epithelial dysplasia was recorded. They could correlate the increased epithelial thickness, especially the presence of a vacuolated surface layer, with the clinical severity. These findings were confirmed by Hirsch, Heyden & Thilander (1982) but they also found an association between the clinical severity and more deeply located cell changes such as mitotic figures, moderate inflammation, oedema and sialadenitis.

The concept of epithelial dysplasia was used by Roed-Petersen & Pindborg (1973) for a disorderly maturation, not involving all layers of the epithelium and comprising 2 of the following features: irregular stratification, hyperplasia of the basal cell layer, drop-shaped rete pegs, increased number of mitotic figures (a few abnormal mitoses might be present), increased nuclear-cytoplasmic ratio, loss of polarity of the basal cells, nuclear hyperchromatism, enlarged nucleoli, keratinisation of single cells or cell groups in the prickle cell layer and loss of intercellular adherence. One case with slight epithelial dysplasia was recorded. At a follow-up examination, another case of snuff-induced leukoplakia had developed a carcinoma. Based on these 2 cases, the authors concluded that 6.2% of snuff-users' lesions were associated with premalignant or malignant changes.

Hirsch, Heyden & Thilander (1982) detected slight dysplastic changes in 18% of the biopsies and they found them in lesions of all four clinical degrees. Concerning the criteria for dysplasia, the authors just refer to WHO definitions without specifying which of the different criteria had been fulfilled.

Frithiof, Anneroth, Lasso et al. (1983), in a study of 21 male snuff-users, recorded 5 cases of mild epithelial dysplasia in the form of drop-shaped rete processes, reduction of cellular adhesion in the basal and spinous cell layers and slight cellular pleomorphism. They concluded that the premalignant significance of the mild dysplasia found in their study was questionable and might be a reactive change due to inflammatory infiltration. Jungell & Malmström (1985) supported this theory in their study on 21 snuff-users among Finnish recruits. They found only one case of mild epithelial dysplasia, consisting of atypical and increased number of mitoses and loss of basal polarity.

Greer, Poulson, Boone et al. (1986) used roughly the same criteria as Roed-Pedersen & Pindborg (1973) to identify dysplasia. Only one case in the total sample of 45 was recorded. This patient had also been a smoker and had used alcohol. They therefore found it impossible to determine whether smokeless tobacco use alone accounted for the dysplasia. In a study by Greer, Eversole, Poulson et al. (1987) on 27 subjects 16 to 81 years old and with regular snuff use for 2-57 years, only minimal or no cytological atypia were recorded. Neither could any atypia be demonstrated by Greer & Schroeder (1988) in 77 tissue samples from patients 16-71 years of age, who had used smokeless tobacco for from 6 months to 50 years.

In the study from 1986, Greer, Poulson, Boone et al. identified koilocytotic changes in 26 patients out of 45. They defined koilocytosis as the presence of a perinuclear clear zone and pyknotic, irregularly-shaped nuclei within the spinous cells of the squamous epithelium. By using PAP immuno-histochemical techniques, they were able to demonstrate human papilloma virus (HPV) capsid antigen in 6 cases. Based on the morphological changes seen in their study, they suggested viral association in a majority of hyperkeratoses associated with smokeless tobacco, warranting investigation of the possibility that HPV may play a primary causal role in the development of epithelial dysplasia or carcinoma of the oral cavity. The authors also suggested a possible synergistic effect between HPV and tobacco which may enhance their persistent "low carcinogenicity" allowing future malignant change in "predisposed" tissue.

In 27 smokeless tobacco users examined by Greer, Eversole, Poulson et al. (1987), the koilocytotic changes were further analysed. Koilocytes were present in 13 biopsies. Nine of them displayed capsid antigen (PAP+) and 5 were positive for HPV structural protein (DNA+) when in situ hybridisation techniques were used. The investigators suggested, based on these findings, that smokeless tobacco-associated leukoplakias were occasionally correlated with HPV infection. However, they also stated that the postulate that assumes progression of these specific smokeless tobacco-induced keratoses to dysplasia or carcinoma needs to be further evaluated. The suggestion from this study was confirmed in a study by Greer & Schroeder (1988). They found that the most common feature in snuff-related changes identified light microscopically was koilocytosis. Koilocytotic changes were present in 60 tissue samples out of 77. Sixteen specimens showed presence of HPV capsid

antigen. However they also stated, in accordance with Greer, Eversole, Poulson et al. (1987), that further investigations are needed to determine whether there is a significant "delay" in expression of viral protein that might be related to the clinical grade of the smokeless tobacco lesion, the length of time the patient has been using the tobacco product, or the type of product.

Kaugars, Mehailescu & Gunsolley (1989) analysed 1,651 cases with a histologically confirmed diagnosis of dysplasia of the oral cavity. They identified 108 patients with a history of smokeless tobacco use. Dysplasia was divided into 4 histological grades: focal mild with only isolated foci of dysplastic features, mild, moderate and severe. The latter 3 categories corresponded to an increasingly greater percentage of the epithelium demonstrating dysplastic features. No mention was made of which histological features were used to define dysplasia. Focal mild dysplasia was recorded in 17.1%, mild dysplasia in 66.7%, moderate in 10.8% and severe in 5.4%. Focal mild or mild dysplasia was thus recorded in about 84% of the cases. The prevalence of dysplastic features in smokeless tobacco induced lesions given in the study by Kaugars, Mehailescu & Gunsolley is contradictory to information given in the studies reported above and also in a publication from Grady, Greene, Daniels et al. (1990), who examined 463 smokeless tobacco users among American professional baseball players. Ninety-eight leukoplakic areas in 92 subjects were biopsied and examined histologically. All lesions were benign and only one case showed mild epithelial dysplasia. This lesion was clinically classified as degree 1 and occurred in a man who had used half a can of Copenhagen snuff a day for 3 years.

Pindborg & Poulsen (1962) published a preliminary report on the influence of snuff upon the connective tissue of the oral mucosa. Histological investigation revealed a thickening of the squamous epithelium and areas of homogeneous tissue at the transition between lamina propria and tela submucosa. These mucosal changes were further examined by Lyon, Poulsen & Pindborg (1964). They reported on 7 men with regular snuff use for 20-50 years. All of them showed epithelial thickening and 4 of them areas of homogeneous tissue, suggestive of amyloid, in the underlying connective tissue. This feature was also recorded by Archard & Tarpley (1972) when analysing biopsies from 3 snuff users. They could not identify the deposits as amyloid, however. Axéll, Mörnstad & Sundström (1976) also identified amorphous

areas in the connective tissue papillae in 9 cases of 114. They suggested that these areas reflected collagen that had been altered by a direct chemical insult from the snuff. Submucosal eosinophilic deposits were demonstrated by Hirsch, Heyden & Thilander (1982) in one of 50 snuff dipper's lesions. Greer, Poulson, Boone et al. (1986) also identified this feature in 2 out of 45 biopsies.

Hirsch, Heyden & Thilander (1982), who examined 50 habitual snuff dippers, reported a sialadenitis and degenerative changes in salivary glands in 42% of the subjects. Gland tissue was present in 74% of the specimens, predominantly in lesions of clinical degrees 3 and 4. The investigators concluded that salivary glands seemed to be subjected to more severe damage than the epithelium and that this may lead to decreased saliva production and hence decreased protection of the epithelium. This conclusion is, however, somewhat contradictory to findings by Jungell & Malmström (1985), who reported a somewhat higher salivary flow among snuff-users than among nonusers. Neither could the findings be confirmed by Greer, Poulson, Boone et al. (1986). In their study including juvenile, adult and geriatric patients, sialadenitis and degenerative changes were only found in 4 out of the 20 biopsies which included gland tissue. Neither could they correlate tobacco-associated salivary gland fibrosis and the clinical degree of a lesion. They suggested that glandular changes might be associated with the tobacco brand.

Reversibility

In 1963 Pindborg & Renstrup reported on the effect of snuff on oral epithelium. Snuff dipper's leukoplakia was recorded in all 12 male patients. One patient discontinued snuff use for 3 weeks while he was in hospital. During this time the clinical appearance of the mucosa returned to normal.

Smith (1972) stated that when snuff was withdrawn, the snuff-induced tissue changes in most instances were reversible regardless of the time that snuff had been used.

Axéll, Mörmstad & Sundström (1976) noted that clinically the mucosa changed its appearance after only a few days without snuff exposure. Six of their 114 patients had stopped snuff-dipping between the first examination and the biopsy session a few days later. They were not able to classify the snuff dipper's lesion of these 6 cases according to a four-point clinical scale. They were therefore placed in a special group called "degree X" and in the histological analyses less pronounced changes were recorded in this group. The clinical findings were further confirmed by Frithiof, Anneroth, Lasso et al. (1983). In their study, all patients were recommended to break the habit of snuffing. In those who did, the mucosa was markedly normalised in structure and colour after one week, and after another week the mucosa was almost perfectly normal. Jungell & Malmström (1985) also found that snuff-induced lesions were clinically reversible. Out of the 48 snuff-users identified in their population of Finnish recruits, 8 had given up the habit 3 weeks before examination. They showed no pathological alterations of the oral mucosa.

The clinical reversibility of lesions associated with snuff use is further supported by a case report from Giunta & Conolly in 1986. They reported on a 33-year-old man who had used snuff 10-12 h daily for 20 years. He showed leukoplakia in the buccal sulcus where the tobacco was placed. The patient was persuaded to stop using snuff for 4 weeks and at the re-examination the leukoplakia had regressed and later it disappeared.

Concluding remarks

Previous and current literature thus seems to be equivocal concerning which consumption factors have the greatest impact on the development of oral mucosal changes associated with snuff use. To what extent the recently introduced portion-bag-packed snuff plays a decisive role in this respect is not known at all. Labelling as well as the classification of the snuff-induced lesions are a cause of controversy. Further, the possible reversibility of snuff-induced changes is not satisfactorily described and the tissue reactions, especially in the sense of dysplasia, are not well understood.

AIMS OF STUDY

The aim of this study was to analyse soft tissue changes associated with the use of two different smokeless tobacco products, loose and portion-bag-packed Swedish moist snuff. In particular, the purpose was

- to record and to compare oral mucosal lesions, clinically and histologically, associated with the two products,
- to record and to compare the prevalence of gingival recessions associated with the two products,
- to analyse the relative importance of various consumption factors on clinical and histological changes found at the site(s) where the snuff was regularly placed,
- to analyse the association between a four-point clinical scale and the histological features,
- to evaluate the tissue response to a change of snuff habit and
- to suggest follow-up routines for snuff-related lesions

METHODS

Examination procedures (Papers I, II, III, IV and V)

All subjects were examined according to a standardised programme. Questions were asked on snuff habits, package form, brand of snuff, years with regular habit, daily consumption of snuff, placement of the quid (at one or more sites) etc. Information was also collected on other current and/or previous tobacco habits, alcohol consumption, general health and medication. Clinical examination was carried out, including registration of changes at the place where the snuff was regularly placed according to a four-point clinical scale suggested by Axéll, Mörnstad & Sundström (1976) (Figs 1-4):

Degree 1 - A superficial lesion with a colour similar to the surrounding mucosa and with slight wrinkling. No obvious mucosal thickening.

Degree 2 - A superficial, whitish or yellowish lesion with wrinkling. No obvious thickening.

Degree 3 - A whitish-yellowish to brown, wrinkled lesion with intervening furrows of normal mucosal colour. Obvious thickening.

Degree 4 - A marked yellowish to brown and heavily wrinkled lesion with intervening deep reddened furrows and/or heavy thickening.

Gingival recessions were registered as snuff-related when the borderline of the vestibular snuff dipper's lesions coalesced with the retracted gingival margin (Fig. 5). All mucosal and gingival changes were photographed in colour.

Histological analysis (Papers II, III, IV and V)

A biopsy was taken from the central part of each changed mucosal area with a 6 mm punch instrument.

The specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin, PAS, rhodamine-B or Congo Red and examined light microscopically including under fluorescent light.

Biopsies showing koilocytosis-like changes were examined immunocytochemically for the presence of HPV-antigen. Epithelial atypia were recorded based on criteria defined by Smith & Pindborg (1978) and the 8 histological features reported by Kramer (1980) to be important discriminators of separate cases with leukoplakia that subsequently developed carcinoma from those that did not.

Statistical methods (Paper I)

Chi-square tests were used to test the differences between frequencies and Student's t-test for the differences between means. Stepwise logistic regression was applied to study the relative risk of developing clinical lesions in relation to package form, exposure data and age.

SUBJECTS

Paper I

The 252 subjects included in the study were recruited from 3 populations: 1) construction workers, 2) shipyard workers, 3) outpatients at the School of Dentistry in Malmö. All of them reported daily snuff consumption for at least the last 3 months and no other current tobacco habit. None had any serious disease and/or medication that might influence the local reaction of the oral mucosa. Loose snuff was used by 184 subjects, mean age 36.0 ± 11.2 years, and portion-bag-packed snuff by 68, mean age 36.9 ± 9.9 years.

To fulfil the aims of the study, specially selected fractions of the total material have been used in accordance with information given below.

Paper II

Biopsies were obtained from all the 252 volunteers. In addition, in order to compare snuff-associated histological tissue changes, 14 matched pairs of loose and portion-bag users were selected. They used the same brand of tobacco and exhibited closely similar patterns of daily snuff exposure.

Paper III

In order to analyse the relative importance of consumption factors on tissue changes, 16 loose snuff users with close to equal daily consumption (h/day, g/day) were selected. They were grouped in 8 pairs with the two individuals of each pair showing the largest possible differences in terms of years of regular snuff use (range differences 14-45 years). The range of consumption of snuff within the eight pairs of loose snuff users was 12.5-33.3 g daily for 2-25 h and they had been regular snuff-takers for 3-50 years.

Four fractions of subjects showing the lowest and highest daily consumption of loose and portion-bag-packed snuff were selected. They included 5 low consumers in each group and 8 loose and 7 portion-bag users with high daily

exposure to snuff. The 5 men with a low consumption of portion-bags used 8 g of snuff or less for no more than 7 h a day and the 7 high consumers used 16 g of snuff or more for at least 13 h a day. The corresponding figures for the loose snuff users were 5 men using 7.1 g of snuff or less for no more than 6 h a day and eight subjects using 25 g of snuff or more for at least 15 h a day.

Paper IV

Among the 252 snuff-takers, 29 loose snuff users exhibited some degree of epithelial changes in addition to what could be generally observed at the histological examination. In order to analyse possible reversibility of these changes, all the 29 subjects were re-examined clinically and histologically after 3-6 months after efforts to make them stop or at least change their snuff habit. The biopsy for microscopical re-evaluation was always taken from the same mucosal area as the initial biopsy.

Five loose snuff users with a daily snuff exposure of at least 25 g for at least 12 h were selected for comparison.

Paper V

In order to analyse the relevance of the four-point clinical scale, 10 consecutive subjects were selected from each of the four "clinical lesion groups" of the total collected material. From loose snuff users were selected all 10 with Degree 1 lesions, 10 out of 33 with Degree 2 lesions, 10 out of 130 Degree 3 lesions and 10 out of 11 with Degree 4 lesions. From portion bag snuff users were selected 10 out of 13 with Degree 1 lesions, 10 out of 31 with Degree 2 lesions and 10 out of 24 with Degree 3 lesions. A total of 70 subjects were thus included since no Degree 4 lesion was encountered among portion-bag users.

RESULTS

Paper I

The 184 (73%) users of loose snuff consumed 23.6 ± 12.2 g/day during 10.8 ± 3.8 h and had done so for 13.1 ± 8.2 years. The corresponding figures for the 68 (27%) users of portion-bag-packed snuff were 11.3 ± 4.9 g/day during 10.3 ± 3.2 h for 3.1 ± 2.5 years.

The distribution according to clinical grading of lesions was as follows: users of loose snuff showed 10 (5.4%) Degree 1 lesions, 33 (17%) Degree 2 lesions, 130 (70.7%) Degree 3 lesions and 11 (6%) Degree 4 lesions. Users of portion-bags showed 13 (19.1%) Degree 1 lesions, 31 (45.6%) Degree 2 lesions and 24 (35.3%) Degree 3 lesions. The higher proportion of clinically more pronounced changes among loose snuff users in comparison with portion-bag users was highly significant ($P < 0.001$).

Among those who used loose snuff, 112 (60.9%) regularly placed the quid at the same site. The corresponding figure for users of portion-bags was 48 (70.6%).

Gingival recessions were found in 44 (17.8%) of 247 subjects. Five users of loose snuff were excluded because they had full dentures. Among users of loose snuff, 42 (23.5%) showed gingival recessions while only 2 (2.9%) cases were found among users of portion-bags. This difference was statistically significant ($P < 0.05$).

The relative influence of some factors, expressed as relative risk (RR), on clinical grading of snuff dipper's lesions and gingival recessions was calculated by stepwise logistic regression. The most important factors for the development of more pronounced clinical changes were the package form ($RR=3.39$) and placement of the quid ($RR=2.9$). Considerably lower relative risks were noted for daily hours of snuff use ($RR=1.13$), grams of snuff used daily ($RR=1.05$), years of regular snuff use ($RR=1.04$) and age of the subject ($RR=1.01$). The factor with the strongest influence on the development of gingival recessions was the package form ($RR=8.71$). Considerably lower risks were noted for placement of the quid ($RR=1.46$), hours of daily snuff use

(RR=1.04), age of the subject (RR=1.03), grams of snuff used daily (RR=1.01) and years of regular snuff use (RR=1.01). The probability values related to relative risk figures were statistically significant ($P<0.05$) for package form, placement of the quid and daily consumption concerning snuff dipper's lesion and for package form concerning gingival recessions.

That the package form is the most important factor for the development of clinically more severe lesions is further illustrated in Figures 6-7. Two subjects, using loose and portion-bag snuff respectively, were matched according to tobacco exposure data. The user of loose snuff showed a clinical Degree 3 lesion while the portion-bag user showed a clinical Degree 1 lesion.

Paper II

Total material

In biopsies from the 252 snuff-users a multitude of structural changes, appearing in different combinations, were identified. The histological features were defined as described below.

Changes in the surface layer - Two different patterns, "type 1 and 2", were recognised. In "type 1", the surface layer had an increased thickness of variable degree, often in combination with vacuolated cells and the chevron type pattern. "Type 2" showed a variable degree of keratinisation as evidenced by an eosinophilic and a more or less continuous rhodamine B stain. Combinations of "type 1 and 2" were also identified. "Type 1" was the feature most commonly encountered.

Atrophy and hyperplasia - This merely refers to the thickness of the epithelium. Hyperplasia was arbitrarily defined as at least 1.5-2-fold increased thickness of normal epithelium. Similarly, the epithelium was defined as atrophic when loss of rete pegs was observed in conjunction with an over-all reduction of the epithelial thickness.

Increased mitotic rate - When more than two mitotic figures could be identified within an epithelial area of approximately 0.08 mm^2 the mitotic rate was considered increased.

Koilocytosis - Vacuolated cells were presumed to be koilocytotic when appearing in small narrow clusters with no obvious connection with otherwise continuous layers of degenerative cells and with a vacuolated cytoplasm appearing as a clear rim around a pyknotic nucleus. Seventeen cases of koilocytosis-like changes were identified. However, no positive HPV-antigen immunoreactivity could be demonstrated in any of these biopsies.

Increased cellular density and basilar hyperplasia - An increased cellular density mainly localised to the basal part of the epithelium was recorded as "basilar hyperplasia". This feature was also recorded when the vacuolisation of epithelial cells in the prickle cell layer was decreased and partly also due to a better dye-affinity of the cytoplasm of these less vacuolated cells.

Variable degrees of a non-specific chronic inflammation in the connective tissue were observed in all cases. Eosinophilic amorphous or hyaline connective tissue changes, staining amyloid negative with Congo Red, could be recorded in 12 cases.

Only comparatively few salivary glands were included in the biopsies, which therefore did not allow a consistent evaluation of gland tissue changes.

Epithelial changes differing from those generally seen as a result of snuff use, as described above, were observed in 29 men. They became the subjects for a follow-up study (Paper IV).

Matched pairs

-loose and portion-bag snuff users with equal daily consumption.

Users of loose snuff showed more pronounced clinical changes, predominantly Degree 3 lesions, accompanied by histological "type 1" changes in all cases but one. Use of portion-bag-packed snuff was predominantly associated with Degree 1 and 2 lesions and comparatively more of "type 2" or only very discrete changes.

All the 28 (14x2) subjects showed some degree of non-specific inflammation. Cases of hyperplasia and cases of increased mitotic rate were evenly

distributed between the two groups of snuff-users. Altogether 4 cases of increased cell density were recorded.

Paper III

Loose snuff use for many years did not per se result in clinical or histological tissue changes which differed significantly from changes seen in subjects with only a few years of loose snuff use. Predominantly clinical Degree 3 lesions were encountered. Clear-cut histological differences between the individual subjects of each separate pair were difficult to identify in the biopsies. The different types of surface changes ("types 1 and 2") were evenly and randomly distributed among the subjects, with "type 1" predominating. Five cases of epithelial hyperplasia were recorded, 4 of them within two matched pairs. A slight difference in mitotic rate between subjects in some of the matched pairs was identified.

High consumption of loose and portion-bag snuff was associated with more pronounced clinical and histological epithelial changes than low consumption. "Type 1" surface epithelial changes and an increased mitotic rate were the predominant histological findings in those with high consumption. It was difficult to identify histological differences between the two snuff habit groups with high daily consumption with the exception of one subject using loose snuff. Among users with low daily consumption, portion-bag packed snuff tended to be associated with less changes than loose snuff.

Paper IV

Twenty-nine loose snuff users who showed histological epithelial changes in addition to what was generally observed were subdivided into 4 groups as follows:

Group 1 comprised 7 subjects. Four of them showed clinical Degree 3 and 3 clinical Degree 4 lesions. On histological analysis, all of them showed a combination of an increased mitotic rate, increased cellular density and loss of cohesion. Eosinophils were recorded in 6 cases. Hyaline, Congo-Red-negative, connective tissue changes, were encountered in 4 specimens.

Group 2 comprised 20 subjects. One subject showed a clinical Degree 2 lesion, 17 clinical Degree 3 and 2 clinical Degree 4 lesions. On histological examination, all of them showed an increased mitotic rate and an increased cellular density.

Group 3 comprised a single subject with a clinical Degree 2 lesion. On histological analysis it showed increased cellular density, eosinophils and hyaline, Congo-Red-negative, connective tissue changes.

Group 4 also comprised one subject, a clinical Degree 2 lesion showing loss of cohesion, increased cellular density and eosinophils in the histological analysis.

At follow-up 16 subjects had stopped their snuff habit at least 3 months before the re-biopsies. Four of these were in group 1, 11 in group 2, and the single subject in group 4. Clinically, all 16 showed a normal mucosa at the site of the re-biopsy, which also showed a normal histological picture.

Another 4 subjects in group 2 had changed to portion-bag-packed snuff. They had reduced the amount of snuff consumed daily but, above all, they did not place the snuff quid at the site from which the initial biopsy was taken. At follow-up all of them had a normal-looking mucosa at the previous biopsy site, a finding which was supported by the histological analysis. At the site where the snuff quid was now placed, they all showed a snuff dipper's lesion although of a lower clinical degree than at the initial biopsy site.

Six individuals, 2 in group 1 and 4 in group 2, had also changed to portion-bags and reduced their daily exposure to snuff. They still placed the quid at the initial biopsy site but also regularly placed it at different sites. Clinically, they all showed a snuff dipper's lesion but of a lower degree (1-2). The histologic examinations showed inflammation and minor epithelial surface changes. However, none of the group 1 or 2 features could be found.

One subject in group 2 only modified his habit slightly. At follow-up he presented with almost the same clinical picture but histologically no increased cell density was recorded. Two subjects, one each in group 1 and 3, did not change their snuff habit. On re-examination they presented with an unchanged clinical and histological picture.

Three of the 5 selected subjects for comparison had either stopped using snuff or changed to portion-bags and, moreover, they placed the quid at another site. On follow-up they showed a normal mucosa at the initial biopsy site, clinically as well as histologically. The other 2 subjects had only modified their habit slightly and showed mucosal clinical and histological changes similar to the initial ones.

Paper V

The histological surface changes were subtle in Degree 1 and became more pronounced with increasing degree. In 3 cases of clinical Degree 1 and 2, a histologically normal-looking mucosa was recorded. A necrotic rhodamine-B-negative surface zone, either alone or in combination with a "type 1" change, was a common finding in all clinical degrees. A "type 2" surface pattern was recorded in about 15% of the biopsies, predominantly in clinical Degree 1 and 2 lesions.

Atrophy, hyperplasia, an increased mitotic rate and increased cellular density were recorded increasingly more often in lesions with a higher clinical degree. Loss of cellular cohesion was only found in Degree 4 lesions.

The histological reaction patterns, with more pronounced epithelial changes related to increasing clinical degree, were similar for the two package forms of snuff.

Differences in tissue response between users of loose and portion-bag snuff were seen especially in Degree 1 lesions. Those lesions associated with use of loose snuff showed more "type 1" surface changes. Further, it should be noted that no Degree 4 lesion was encountered among portion-bag snuff users.

In the connective tissue, non-specific inflammation of varying degree was found in all cases but connective tissue hyalinisation close to the epithelium was predominantly recorded in Degree 4 lesions.

GENERAL DISCUSSION

Selection of material

For practical reasons, it would not be feasible to carry out this study in a general population. Instead, well-defined subpopulations were selected for the present study. The major part of the subjects were construction workers aged 20-40 years. However, the age-range for the total material was 17-80 years, thus including both rather young and elderly people. This age distribution differs somewhat from that of a report on snuff-users in Sweden from the National Central Bureau of Statistics (1985), where the age distribution of snuff-users in Sweden is given. It shows a bimodal distribution, with high proportions around the ages of 16-34 years and 75-84 years. The lowest frequency of snuff use is found in the age-group 45-54 years.

However, there are at present no convincing data indicating a considerable difference in tissue response related to age of snuff-users (Mörnstad, Axéll & Sundström 1989). Moreover, recent studies have indicated that daily exposure is a more important factor in this respect (Wolfe & Carlos 1987, Grady, Greene, Daniels et al. 1990). Further, the clinical and partly the histological analyses carried out in this study have focused on comparison between two different package forms, loose and portion-bag snuff. The subjects in both groups have been recruited through a parallel procedure.

Clinical grading

A clinical subdivision of snuff dipper's lesion with well-described criteria is valuable not only for evaluation and analysis of materials but also for the follow-up of patients and comparison between different studies. Axéll, Mörnstad & Sundström (1976) suggested a four-point scale for subdivision of clinical mucosal changes recorded at the site where the snuff quid was regularly placed. This scale was applied when recording changes in the present study (Paper I). The inter-examiner agreement between the author and Axéll in classification was 95%, suggesting that the criteria are acceptably reliable diagnostic tools for describing various degrees of snuff dipper's lesion in users of Swedish moist snuff.

Greer & Poulson (1983) modified the four-point scale to a three-point scale ~~because they could not identify any Degree 4 lesion in their material.~~ They believed that this was due to the fact that only subjects with snuff use for 4 years or less were included in their study. However, in a study from 1986 on subjects with up to 36 years of snuff use, the same three-point scale pattern was recognised. The daily exposure to snuff, expressed as minutes per day, given in the studies by Greer & Poulson (1983) and Poulson, Lindemuth & Greer (1984) was much lower than among subjects in this study. This might partly explain why no clinical degree 4 lesion was encountered in their material. This suggestion is further supported by the fact that the clinical Degree 4 lesions recorded by Axéll, Mörnstad & Sundström (1976) and by Hirsch, Heyden & Thilander (1982) were seen among subjects with comparatively high daily exposure.

Histology

The histological patterns of snuff-induced lesions described in this study (Paper II) are largely in agreement with those in previous publications in as much as they show an increased thickening of the surface epithelial layer with vacuolisation, frequently in combination with a chevron-type pattern (Pindborg & Renstrup 1963, van Wyk 1965, Roed-Petersen & Pindborg 1973, Axéll, Mörnstad & Sundström 1976, Pindborg, Reibel, Roed-Petersen et al. 1980, Hirsch, Heyden & Thilander 1982, Jungell & Malmström 1985, Greer, Poulson, Boone et al. 1986). In the present study, these histological features have been labelled "type 1". However, contradictory to most other studies, the thin eosinophilic surface zone associated with "type 1" changes or sometimes recorded separately was not keratinised. It was interpreted as a coagulative necrosis due to chemical etching by the alkaline snuff. Occasionally a keratinised surface layer was recorded, in this study labelled "type 2", indicating a physiological reaction of keratinisation in this normally unkeratinised area of the oral mucosa. This might tentatively be an expression of a protective reaction comparable to what can be found as a response to mechanical trauma (frictional white lesions). "Type 2" surface changes were more commonly encountered in lesions of a lower clinical grading. Degree 1 and 2 lesions were more common in subjects with a lower daily exposure to snuff. It may be suggested that when the mucosa is not exposed to snuff throughout the day a mixed tissue reaction of injury and

repair may prevail. These findings confirm suggestions by Axéll, Mörnstad & Sundström (1976), who also observed this association between changes of various clinical degrees and the keratinisation process. They also found a difference in epithelial thickness between keratinised and non-keratinised areas in snuff dipper's lesion but did not analyse it further.

The presence of amorphous changes in the connective tissue was only recorded in a few cases (Paper II). Earlier studies (Pindborg & Poulson 1962, Lyon, Poulsen & Pindborg 1963, Archard & Tarpley 1972, Axéll, Mörnstad & Sundström 1976) reported this histological feature in rather high frequencies in snuff-induced changes. However, later studies (Hirsch, Heyden & Thilander 1982, Greer, Poulson, Boone et al. 1986) have reported amorphous changes as decreasingly present in biopsies from snuff-users. This might support the theory that hyalinisation is a result of denaturation of the proteins due to a chemical trauma and correlated to specific brands and, especially, the pH value of the snuff.

Detailed criteria were applied for atrophy, hyperplasia, increased mitotic rate, koilocytosis, increased cellular density and basilar hyperplasia (Paper II). This has been done partly to facilitate comparison between changes related to different products and to make it possible to relate the clinical grading system to the histological picture in snuff dipper's lesion as reported in Papers II, III, IV and V.

Although there was no clearcut difference between findings in specimens from lesions of each separate clinical degree, either clinically or histologically, a rather consistent over-all pattern of tissue changes was recorded. In lesions of lower clinical degree no or only a slightly increased thickening of the surface layer was recorded, becoming more marked with increasing clinical degree (Paper V). This is in accordance with findings in previous studies (Axéll, Mörnstad & Sundström 1976, Hirsch, Heyden & Thilander 1983). More pronounced epithelial changes such as increased mitotic rate, increased cellular density and loss of cohesion were also more commonly recorded in lesions of higher clinical degree. Hirsch, Heyden & Thilander (1982) also found an association between the clinical degree and some deeply located mucosal changes but they concluded that marked histomorphological changes, such as dysplasia, could not be predicted by the clinical degrees.

However, the present study indicates that a relationship frequently exists ~~between clinical lesions classified according to the fourpoint grading system~~ of snuff dipper's lesion and the histological epithelial changes (Paper V). It should be emphasised, however, that considerable individual variations do exist.

Seventeen cases of koilocytosis-like changes were identified (Paper II). Almost the same lightmicroscopic criteria were applied for this feature as those used by Greer, Poulson, Boone et al. (1986). However, in contrast to their findings, HPV-antigen could not be immunohistochemically demonstrated in any of the biopsies. Six specimens with the most pronounced koilocytosis-like changes have been further analysed with the in-situ DNA hybridisation technique but none of them was positive (isotypes nos. 2, 6, 11, 16, 18; S Syrjänen, personal communication).

In a study by Greer, Schroeder & Crosby (1988), koilocytes were even more commonly encountered than other histological features such as chevron keratinisation and vacuolisation in the superficial layers. To what extent differences in products, consumption parameters and laboratory procedures or inter-examiner differences can explain the discrepancy between their results and ours can at present only be speculated upon. Collaboration concerning future analyses is advisable and could be of great value.

The detailed definitions of different histological parameters also turned out to be of great value in a follow-up of a selected material of biopsies from 29 subjects with snuff dipper's lesions (Paper IV). All these specimens exhibited epithelial alterations different from or in addition to what was subjectively found to predominate in snuff dipper's lesion as defined in Paper II. The changes recorded were various combinations of an increased mitotic rate, an increased basal cell density and loss of cell cohesion. The criteria for these features have been set by Smith & Pindborg in their classification of oral epithelial atypia (WHO 1969).

On re-examination, all 20 of the 29 subjects who either stopped their snuff habit completely or changed the site of placement of the quid showed a normal mucosa clinically as well as histologically (Paper IV). Thus, epithelial changes representing some of the parameters used to define dysplasia turned out to be completely reversible. Seven subjects modified their snuff

use in various ways. They changed product, placed the snuff quid at more than one site and reduced their daily exposure to snuff. All of them still showed a clinical lesion at follow-up, but of a lower degree. Reduced epithelial changes were recorded in all biopsies, even in subjects who just reduced the snuff exposure of the oral mucosa, probably reflecting a process of repair. This is also indicated by the fact that more pronounced epithelial changes could not be found at all at follow-up.

Epithelial dysplasia has been considered an irreversible lesion that might proceed to a malignancy. However, Kramer (1980) concludes "that as a mirror of the underlying biologic changes that lead to malignancy, epithelial dysplasia is a very imperfect mirror". Further, at an international seminar on oral leukoplakia and associated lesions related to tobacco habits (Axéll, Holmström, Kramer, Pindborg & Shear 1983), it was emphasised that follow-up studies are mandatory to test a revised assessment of epithelial dysplasia. One important conclusion from the follow-up in this study (Paper IV) was that the recorded snuff-related changes were reversible. They seem to represent a well-defined set of changes associated with the regular use of Swedish moist snuff. Correct identification of these changes, which are interpreted as reactive, is important to avoid errors in diagnosis and treatment.

Consumption parameters

In the present study (Paper I), the influence of different consumption parameters on the development of clinical snuff-induced lesions was calculated by means of stepwise logistic regression analysis. The conclusion was that daily exposure has the largest impact on the clinically recorded snuff-induced changes. This was clearly supported by a histological analysis based on a selected material (Paper III). Despite large differences in years of regular snuff use, no considerable differences could be found on histological evaluation of snuff-users with comparable daily exposure.

The importance of daily exposure to snuff was further emphasised in the analysis of subgroups with low and high daily consumption (Paper III). Clinically, lesions with a lower degree were more commonly recorded among low than among high consumers. Histological analysis showed more pronounced tissue reactions (evidence of surface etching with development

of "type 1" change, increased mitotic rate) among subjects with high daily exposure to snuff, in contrast to those found among subjects with a low daily exposure. The relative importance of daily exposure to snuff is confirmed in a study by Grady, Greene, Daniels et al. (1990). They found that the amount of smokeless tobacco, expressed as hours of daily snuff use, was the only independent predictor of the severity of the clinical lesion. The same statistical multivariate analysis using logistic regression was used as in the present study.

In previous studies by Roed-Petersen & Pindborg (1973) and Hirsch, Heyden & Thilander (1985), a simple cumulative index was used for the corresponding calculations. The total exposure was calculated, based on the assumption that number of years with the snuff habit and daily exposure to snuff in hours and grams of snuff consumed a day had equal influence on the tissue response. However, judging by the present findings, this index might not truly reflect the tissue response in relation to different consumption factors.

Package form

Users of portion-bag snuff showed predominantly snuff dipper's lesion of clinical Degrees 1 and 2 (about 65%) while users of loose snuff showed predominantly lesions of clinical Degrees 3 and 4 (about 75%)(Paper I). No clinical Degree 4 lesion was encountered among portion-bag users. One explanation for this could be that the pattern of placement of the snuff quid differed. However, placing the quid in one site was even more frequent among portion-bag users than among loose snuff users. Further, stepwise logistic regression calculation showed that by far the most promotive factor for the development of clinically more severe lesions was the package form.

There were some differences in snuff exposure data between those users of the two package forms with clinical Degree 2 and 3 lesions (Paper I). Loose snuff users consumed about twice the amount of snuff, in grams a day, as portion-bag users. This can probably be explained by the fact that a portion-bag of snuff contains 0.5 or 1 g while a pinch of loose snuff in most cases contains 1-2 g. Further, loose snuff users also tend to expose a larger area of the mucosa to snuff. Lesions recorded in loose snuff takers also mostly

involve a larger vestibular area adjacent to a greater number of teeth than lesions recorded in portion-bag users.

Differences in years of regular snuff use between users of the two products do not seem to be a plausible explanation for the different clinical tissue response either. Stepwise logistic regression analysis showed that the duration of the habit had comparatively little influence on the development of more severe clinical lesions. This finding was confirmed in the histological analysis of a selected material (Paper III).

The consumption factor with the greatest impact on the severity of snuff dipper's lesion, hours of daily snuff use, was very similar among loose and portion-bag users (Paper I). When evaluating subgroups with low daily snuff consumption, portion-bag-packed snuff turned out to be related to less histological changes than loose snuff. However, among those with a high daily consumption histological differences were difficult to identify (Paper III).

Histologically different epithelial surface changes were identified within matched pairs of users of the two package forms (Paper II). Loose snuff users showed more "type 1" changes and portion-bag users showed more "type 2" changes. However, with increasing clinical degrees, the changes recorded among portion-bag users tended to resemble the histological pattern of loose snuff users (paper V).

The most important difference in tissue specimens from users of the two package forms of snuff is that some of the more pronounced histological parameters, e.g. loss of cohesion, were not recorded among portion-bag users (Paper V). Because of that, no portion-bag users were included in the follow-up material (Paper IV).

One explanation for the different tissue reactions might be a difference in pH value of the snuff in the two package forms. The same tobacco is used in the production of loose and portion-bag snuff. However, at storage the pH value in portion-bag-packed snuff tends to decrease from about 8.7 to 8.2 while loose snuff almost maintains its pH value (T Ringberger, personal communication). The importance of the pH value has been indicated by Grady, Greene, Daniels et al. (1990). Among the 19 subjects in their study who used a smokeless tobacco product with the low pH of 6.0, only one

showed a clinically visible lesion and then of Degree 1-2. They also discussed the possibility that many substances of the snuff quid might influence the tissue reaction.

Gingival recessions

Van Wyk was the first to report on gingival recessions in snuffusers. Greer & Poulson (1983) used criteria for the diagnosis of gingival recessions that are largely in accordance with those used in this study. They discussed whether long-term use might result in more frequent gingival recessions but no systematic evaluation was made. No other author has discussed the influence of different consumption factors on the development of snuff-induced gingival recessions. Rather the connection between snuff use and gingivitis has been discussed. Offenbacher & Weathers (1985) concluded that use of smokeless tobacco was a significant risk factor for the development of gingival recessions in individuals with co-existing gingivitis. However, these results are somewhat contradictory to findings by Mod  r, Lavstedt & Ahlund (1980), who suggested that properties of snuff may directly influence the gingival tissue and cause gingivitis. This is also in agreement with suggestions by van Wyk (1965).

The present study (Paper I) showed a lower prevalence of gingival recessions among portion-bag users (2.9%) than among loose snuff users (23.5%) In fact, the only consumption factor related to a significantly increased risk was the package form of the snuff. The low prevalence of gingival recessions among portion-bag users might be due to less contact between the snuff quid and the gingival margin and also to the avoidance of the mechanical trauma from tobacco particles because tobacco in portion-bag snuff is wrapped in a sachet of a non-woven paper-like material.

In the follow-up material (Paper IV), 6 out of 28 subjects (one was a full denture wearer), corresponding to about 21 %, showed gingival recessions. Four of these stopped using snuff. At follow-up they had gingival tissue of normal texture and colour. However, the loss of gingival tissue, with the necks of the teeth exposed, was recorded to be of the same extent as at the initial examination.

Labelling of snuff-induced lesions

In clinical work and research, diagnostic labels are helpful tools provided that they are carefully described with clear, easily understandable criteria and also that they are generally accepted. Concerning snuff-induced changes, different diagnostic labels have been used for the oral mucosal changes at the site where the tobacco is held. Depending on the contents of the snuff quid, these changes may show varying clinical and/or histological alterations. It would be of advantage if such a "quid" lesion could be recorded as a separate entity provided that clinical criteria and the type of contents, e.g. dry or moist snuff, pH-value, package form and specific additives, are carefully described.

Definitions and guidelines for oral leukoplakia and associated lesions related to tobacco habits were given in a report from an international seminar on oral leukoplakia and associated lesions related to tobacco habits (Ax  ll, Holmstr  p, Kramer, Pindborg & Shear 1983). A complete description of leukoplakia should comprise aetiological, clinical, topographical and histological characteristics. Whitish patches or plaques which are thought to be the result of the use of tobacco should be listed as tobacco-associated leukoplakias, with the exception of "smoker's palate". It was also pointed out at the seminar that leukoplakia is a precancerous lesion.

In the present study, it was found that snuff dipper's lesion is not always whitish (Paper I). This is in accordance with Kaugars, Mehalescu & Gunsolley (1989), who reported partially or completely red lesions in 18.1% of their total material of 108 patients with a history of smokeless tobacco use.

Further, the snuff-induced lesion associated with Swedish moist snuff turned out to be reversible and the changes are interpreted as reactive (Paper IV). This is in agreement with a suggestion by Holmstr  p at the international seminar referred to above. He proposed that reversible lesions may be regarded as physiological reactions to trauma such as tobacco.

Based on the findings in this study, it is suggested that a "quid lesion" should preferably be recorded as a separate entity and not included among leukoplakias for the purpose of follow-up of the development of that specific quid lesion. The suggested label for oral mucosal changes associated with use of snuff is snuff dipper's lesion (Paper V).

CONCLUSIONS

Clinical snuff-related changes of the oral mucosa were less pronounced among those subjects who used portion-bag-packed snuff than those who used loose-packed snuff.

A lower prevalence of gingival recessions was recorded among portion-bag snuff users than among loose snuff users.

Two major histological patterns, "types 1 and 2", were identified, based on changes in the epithelial surface layer. Loose snuff users showed predominantly more "type 1" changes while portion-bag users showed comparatively more lesions with the "type 2" pattern or only discrete changes.

The number of years of snuff use did not per se influence the severity of the tissue changes of the oral mucosa.

Low daily consumption, in terms of hours using snuff daily and grams of snuff used daily, was related to less pronounced clinical and histological changes than high daily exposure to snuff.

Among subjects with a low daily snuff exposure, portion-bag users showed less pronounced changes than loose snuff users. This difference was, however, not found in the high exposure groups.

A four-point scale seemed relevant since Degree 4 lesions were only seen after use of one of the products (loose snuff). Further, non-typical histological features were recorded more frequently with increasing clinical degree.

Clinical snuff-related mucosal changes showed reversibility to normal conditions after cessation of snuff use.

Moderate as well as more pronounced histological snuff-induced changes, including some of the parameters used to define dysplasia, showed reversibility after cessation of snuff use.

Loose snuff users who reduced their daily exposure and/or changed to portion-bag snuff exhibited less pronounced clinical and histological changes at follow-up.

Based on these findings, it is suggested that

- snuff-users should be given thorough information on the association between their mucosal changes and their snuff habit, including advice to stop or to modify the habit
- biopsies should be taken from all Degree 4 lesions and from lesions which show lack of reversibility in spite of a changed snuff consumption pattern
- subjects with snuff dipper's lesion should be followed up on a regular annual basis with clinical documentation, preferably including colour photographs, in order to record alterations in the appearance of the lesion

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Clinical appearance of lesions associated with the use of loose and portion-bag packed Swedish moist snuff: a comparative study

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Andersson G, Axéll T. Clinical appearance of lesions associated with the use of loose and portion-bag packed Swedish moist snuff: a comparative study. *J Oral Pathol Med* 1989; 17: 000-000.

The aim of this study was to register and compare clinical oral mucosal lesions and gingival recessions associated with the use of two different smokeless tobacco products, loose snuff and portion-bag packed snuff. Selected for the study were 252 men (mean age 36.3 yr) of whom 184 (mean age 36.0 yr) used exclusively loose snuff and 68 (mean age 36.9 yr) exclusively portion-bag snuff. Oral mucosal lesions were registered according to a four-grade clinical scale. There was a significantly larger proportion of less pronounced lesions, Degrees 1 and 2, among the users of portion-bag snuff compared with the users of loose snuff. This was also valid when differences in consumption data were considered. Smokeless tobacco-associated gingival recessions were found in 42 (23.5%) subjects among the users of loose snuff and in 2 (2.9%) subjects among the users of portion-bag snuff. The results of this study support previous preliminary assessments that clinical changes of the oral mucosa and the gingival margin are less pronounced among those who use portion-bag snuff than among those who use loose snuff.

Key words: gingival recessions; mucosal lesions, oral; portion-bags; snuff; tobacco, smokeless.

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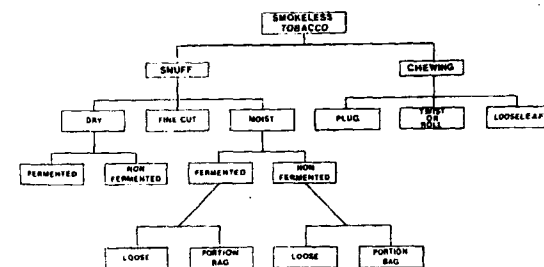


Fig. 1. Classification of smokeless tobacco.

Oral mucosal changes associated with the use of snuff have been described in many studies (1-11). In a survey on the prevalence of oral mucosal lesions in Sweden, a well-recognized mucosal reaction, snuff dipper's lesion, was com-

monly registered in the area where a quid of moist snuff was regularly placed (12). This lesion has also been referred to as leukoplakia (1, 4). In a study on premalignant changes in Danish snuff-induced oral leukoplakias it was

pointed out that not all snuff brands possess the same chemical composition which might explain reported differences in tissue changes (4). The severity of the clinical appearance of snuff dipper's lesion may be related to the hours of daily snuff use, the amount used daily, duration of snuff habit and brands used (6, 7).

The prevalence of gingival recessions associated with the use of smokeless tobacco has been reported in a few studies (13-15). The risk that such users run of developing these recessions has been estimated to be nine-fold greater than that of non-users, provided gingivitis was present (15).

During the last decade consumption of snuff has increased steadily in Sweden, especially among young people. Concomitantly risks for the development of oral cancer and other deleterious side effects have been widely discussed (11, 16-18).

Snuff is manufactured in many differ-

Table 1. Age distribution and some snuff exposure data in different fractions of a construction worker population ($n = 569$).

	Fractions of population				
	Did not answer $n = 109$	Did not want to attend $n = 67$	Mixed habits $n = 215$	Excluded at visit $n = 13$	Finally included $n = 165$
Mean age, yr	32.4±10.4	38.8±13.2	38.0±10.9	36.5±10.0	36.7±10.6
Age range, yr	19-65	21-68	21-74	20-50	18-66
Years with regular snuff habit	10.0± 7.5	10.2± 8.8	10.2± 9.1	11.2±11.4	10.9± 8.4
Grams of snuff used daily	18.8±13.6	15.7±13.4	12.1± 7.9	20.1±13.4	21.3±12.5

Table 2. Age and snuff exposure data.

	Product		
	Loose snuff $n = 184$	Portion-bag snuff $n = 68$	Total $n = 252$
Mean age, yr	36.0±11.6	36.9±9.9	36.3±11.2
Range, yr	19-80	17-66	17-80
Hours of daily snuff use	10.8± 3.8	10.3±3.2	10.6± 3.6
Grams of snuff daily	23.6±12.2	11.3±4.9	20.3±12.0
Years with regular snuff habit	13.1± 8.2	3.1±2.5	10.4± 8.4

ent forms (Fig. 1). Loose snuff is marketed in containers made of paper or aluminium. Usually a pinch of 1-2 g snuff is formed with the fingers and placed in the mouth. In Sweden the overwhelming majority of users place the quid in the vestibular area inside the upper lip.

About 10 yr ago a new smokeless tobacco product, the portion-bag packed snuff, was introduced on the Swedish market. Portions of 0.5 or 1 g snuff are wrapped in bags made of non-woven paper-like material not dissolving in saliva but permitting diffusion. The sales figures have increased steadily and comprised about 10% (470 tons) of the total smokeless tobacco sold in 1987 (19). This product has then been introduced in several other countries.

Oral mucosal lesions associated with the use of loose snuff have been described in several studies, while no report has been published about whether portion-bag snuff causes oral mucosal changes and, if it does, the nature of such changes. As severe local changes may possibly be associated with the use of snuff, it seems worthwhile to investigate lesions related to this new product.

The aim of the present study was to register clinical changes in the oral mucosa and the gingival margin associated with the use of Swedish non-fermented moist snuff and to compare those clinical changes found among users of loose and portion-bag snuff.

Material and methods

Sampling procedure. Recruited for the study were individuals from three populations: 1) construction workers; 2) shipyard workers; and 3) outpatients at the School of Dentistry in Malmö.

A health screening organization for construction workers was established in 1969. Each person within this organization is called for examination every second year. The frequency of participation is about 75%. At the examination questions about tobacco habits are included. About 22% have referred to daily consumption of snuff. Up to 1987 about 300,000 members have been examined throughout Sweden. In the south of the country (Skåne/Scania), where the present study was undertaken, about 3,000 members are examined every year. Of these, 569 consecutive habitual snuff users examined in 1986 were invited to have a check of the condition of their oral mucosa.

At a shipyard (Kockums, Malmö) snuff users were offered a check of their oral mucosa. A total of 43 attended.

Fifty-four patients were recommended by others (dentists, other participants, acquaintances) to ask for a check up at the Department of Oral Surgery and Oral Medicine at the School of Dentistry in Malmö.

Inclusion and exclusion criteria. Included in the study were all who had no

other tobacco habit than snuff and who reported on daily snuff consumption for, at least, the last 3 months. Excluded was anyone with serious disease and/or medication that might influence the local reaction of the oral mucosa.

Non-participants. Two hundred and fifteen of the construction workers had mixed tobacco habits and were excluded, 67 did not want to attend, and 109 did not answer; 178 came for examination. Data of age and snuff consumption in this fraction of studied population are shown in Table 1.

A total of 275 individuals from the three populations attended for examination. At their first visit 23 were excluded after further checking of inclusion and exclusion criteria: 10 people used cigarettes as well as snuff, four used loose as well as portion-bag packed snuff, two had stopped using smokeless tobacco when they came for the examination, one was excluded because of heavy treatment with steroids and four were women. Two further subjects were excluded because the photographs taken were not adequate.

Included subjects. Finally included in the study were 252 subjects. Their mean age was 36.3 ± 11.2 yr. Loose snuff was used by 184, mean age 36.0 ± 11.6 yr, and portion-bag snuff was used by 68, mean age 36.9 ± 9.9 yr.

Examination procedure. All subjects were examined during 1986-87 according to a standardized program. An interview was performed including questions on snuff habits, e.g. package form, brand of snuff, years with habit, daily consumption of snuff, placing the quid at one or more sites. Age and some snuff exposure data are given in Table 2. Information was also collected on other and/or previous tobacco habits, consumption and frequency of wine and/or liquor. Questions were also asked on previous and/or present diseases and medication.

A thorough clinical examination was carried out. Lesions on the site(s) where snuff was regularly placed were registered according to the following four-grade clinical scale suggested by Axéll *et al.* (6).

Degree 1 - A superficial lesion with a color similar to the surrounding mucosa and with slight wrinkling. No obvious mucosal thickening.

Degree 2 - A superficial, whitish or yellowish lesion with wrinkling. No obvious thickening.

Degree 3 - A whitish-yellowish to brown, wrinkled lesion with interven-

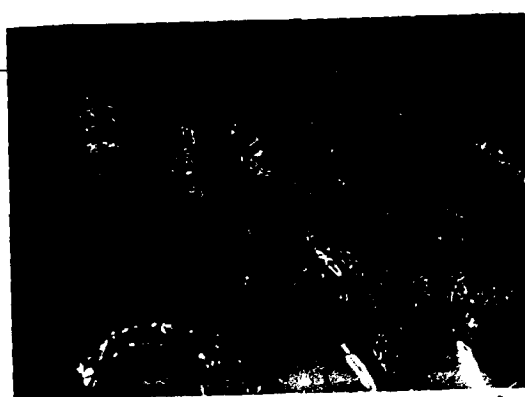


Fig. 2. Snuff-related gingival recession at maxillary left central incisor. Note confluence between recession and snuff dipper's lesion.

ing furrows of normal mucosal colour. Obvious thickening.

Degree 4 - A marked yellowish to brown and heavily wrinkled lesion with intervening deep reddened furrows and/or heavy thickening.

Gingival recessions were also registered. To be registered as a snuff-related recession, the borderline of the vestibular snuff dipper's lesion should be in contact with the retracted gingival margin (Fig. 2).

All mucosal and gingival lesions were photographed in color using a Nikkormat camera with 100 mm macro lens and an extension ring permitting a registration of 1:1. Light was from a Minolta ring flash, the film was Kodachrome 64.

Most of the examinations were carried out by one of the authors (GA) and a few (about 15%) by two other examiners after calibration. After collecting the total material, coded intra-oral photographs were evaluated and classified by the other author (TA), who had no access to information about the tobacco habits of the subjects.

The final clinical grading was based on the initial registration and the analysis of the photographs. Inter-examiner agreement in the classification was about 95%.

Statistical methods - Chi-square tests were used to test the difference between frequencies and student's *t*-test for the differences between means.

Stepwise logistic regression was applied to study the relative risk for developing clinical lesions in relation to package form, exposure data and age.

Results

Loose and portion-bag packed snuff was used by 184 (73%) and 68 (27%) persons, respectively. Snuff-exposure data are given in Table 2. Loose snuff and portion-bag snuff were, on average, kept in the mouth for about the same number of hours daily. However, greater daily amounts of loose snuff were used, and had been used for more years than portion-bag snuff. Table 3 shows exposure data for users of loose and portion-bag snuff related to clinical grading. Clinical grading of lesions is shown in Table 4. Users of loose snuff had a higher proportion of Degree 3 and 4 lesions ($P < 0.001$). Degree 4 lesions were encountered only in persons using loose snuff.

Clinical grading of lesions by snuff brands is shown in Table 5; six brands were used by 232 people (92.1%), the remaining 20 used eight different brands. The average scores for clinical grading were lower for the portion-bag brands compared with the corresponding loose variants. For the brands General and Ettan these differences were statistically significant ($P < 0.001$ and $P < 0.05$, respectively).

Among those who used loose snuff, 112 (60.9%) regularly placed the quid at the same site. The corresponding figure for users of portion-bag snuff was 48 (70.6%).

Table 6 shows the relative risk of the influence of some factors on the development of more severe clinical lesions. The most important factor for increasing risk was the package form, followed by placing of the quid, daily hours of use, amount, duration of regular snuff habit and age of the subject.

Information on previous tobacco habits was available for 241 subjects, 179 loose snuff users and 62 users of portion-bags. Only four users of loose snuff reported that they had earlier used another smokeless tobacco product while 36 of the portion-bag users had previously used loose snuff. Previous smoking habits were reported by 103 (58%) users of loose snuff and 24 (39%) portion-bag users.

Whether the pattern of previous smoking habits had influenced the differences in clinical gradings between users of the two package forms of snuff was tested. Even when stratifying by previous smoking habits there was still a significant difference between users of loose snuff and portion-bag snuff in the distribution of clinical grading ($P < 0.001$).

Wine and/or liquor consumption was reported to be rather infrequent, once a week or less in both groups of snuff users. Ten (5.4%) users of loose snuff and 6 (8.8%) users of portion-bag snuff consumed wine or liquor twice a week. No participant referred to daily consumption of wine or liquor.

Gingival recessions were found in 44 (17.8%) of 247 subjects. Five users of loose snuff were excluded because of full upper and lower dentures. Among users of loose snuff 42 (23.5%) subjects showed gingival recessions while only 2 (2.9%) cases were found among users of portion-bag snuff ($P < 0.05$). Table 6 shows the relative risk factors for the development of gingival recessions. The factor with the highest relative risk (8.71) was the package form.

Discussion

The material for this study was collected from three recruitment categories. Data available on one of the groups, the construction workers, permit some assessment of representativity. Table 1 shows the material for this evaluation. There are minor differ-

Table 3. Cross tabulation of clinical grading versus exposure data of loose and portion-bag snuff.

	Clinical degree								
	Loose				Total n = 184	Portion-bag*			Total n = 68
	1 n = 10	2 n = 33	3 n = 130	4 n = 11		1 n = 13	2 n = 31	3 n = 24	
Age, yr	40.2±8.9	34.0± 9.9	36.0±12.0	38.5±13.7	36.0±11.6	37.2±14.1	35.9±7.6	38.1±10.4	36.9±9.9
Hours of daily snuff use	7.1±4.5	9.1± 4.1	11.3± 3.4	12.2± 3.5	10.8± 3.8	8.4± 3.6	10.5±2.9	11.1± 2.9	10.3±3.2
Grams of snuff daily	10.6±6.7	19.3±10.4	24.9±11.8	32.6±13.6	23.6±12.2	10.8± 3.8	10.7±4.8	12.4± 5.5	11.3±4.9
Years with regular snuff habit	10.5±5.7	10.3± 6.0	13.9± 8.8	14.3± 6.7	10.4± 8.4	4.3± 3.4	2.8±1.9	3.0± 2.6	3.1±2.5

*No clinical Degree 4 lesion was encountered among users of portion-bag snuff.

Table 4. Cross tabulation of clinical grading versus package form of snuff.

Package form	Clinical degree									
	1		2		3		4		Total	
	n	%	n	%	n	%	n	%	n	%
Loose snuff	10	5.4	33	17.9	130	70.7	11	6.0	184	100
Portion-bag snuff	13	19.1	31	45.6	24	35.3	—	—	69	100
Total	23	9.1	64	25.4	154	61.1	11	4.4	252	100

Table 5. Cross tabulation of clinical grading versus brands of snuff.

Clinical degree	General loose	General portion-bag	Grosvnus loose	Grosvnus portion-bag	Ettan loose	Tre Ankare* portion-bag	Row total	All brands
1	5	7	3	2	—	4	21	23
2	17	13	10	8	1	8	57	64
3	94	10	16	5	9	9	143	154
4	10	—	1	—	—	—	11	11
Total n	126	30	30	15	10	21	232	252
Total %	50.0	11.9	11.9	6.0	4.0	8.3	92.1	100
Arithmetical mean of clinical gradings	2.87	2.10	2.50	2.20	2.90	2.24	2.62	2.61

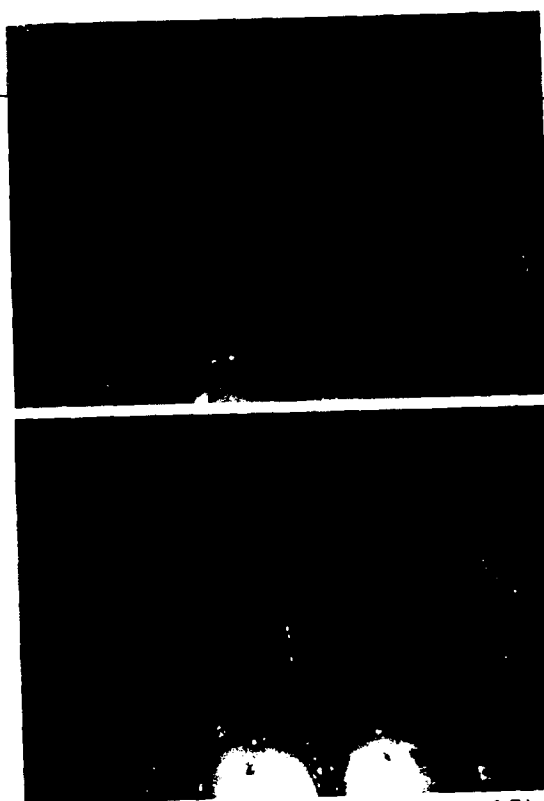
*The portion-bag packed form of Ettan.

ences between included and excluded subjects. Data of years with habit and daily grams of snuff used are somewhat higher within the finally included group, but very similar to those of the total material (Table 2). As could be expected, daily consumption of snuff was lower in the group with mixed habits, supporting the validity of collected information on tobacco habits.

The characteristics of snuff dipper's lesions used in this study were compatible with criteria of the four-grade clinical scale used in previous studies (6, 7). The clinically more severe lesions, Degree 3 and 4, were significantly more prevalent among users of loose snuff than among users of portion-bags. One explanation for this could be that the pattern of placing the snuff quid dif-

Table 6. Relative risk of the influence of some factors on clinical grading of snuff dipper's lesions and on gingival recessions calculated by stepwise logistic regression.

Factor	Snuff dipper's lesion		Gingival recession	
	Relative risk	P-value	Relative risk	P-value
Package form (Loose snuff vs portion-bag snuff)	3.39	0.010	8.71	0.009
Exposure data				
Placing the quid (one vs more sites)	2.91	0.002	1.46	0.284
Hours of daily snuff use (1 h difference)	1.13	0.018	1.04	0.471
Grams of snuff daily (1 g difference)	1.05	0.018	1.01	0.652
Years with regular snuff habit (1 yr difference)	1.04	0.149	1.01	0.677
Age (1 yr difference)	1.01	0.774	1.03	0.141



Figs. 3-4. Fig. 3. Man, 34 yr, showing snuff dipper's lesion clinical Degree 3. Tobacco exposure data: loose snuff of brand Ettan used daily for 10 yr, 15 g daily over 10 h. Until 5 yr ago he smoked 10-15 cigarettes daily. Fig. 4. Man, 43 yr, showing snuff dipper's lesion clinical Degree 1. Tobacco exposure data: portion-bag snuff of brand Tre Ankare used daily for 10 yr, 16 g daily over 10 h. Until 10 yr ago he smoked 30 cigarettes daily.

fered. However, placing the quid on one site was rather more frequent among users of portion-bag snuff.

There were some differences in snuff exposure data between the groups. While daily hours of snuff use were very similar, users of loose snuff consumed a greater daily amount of snuff and had used snuff for a considerably longer period. These differences are not surprising as a portion-bag of snuff contains 0.5 or 1 g, while a pinch of

loose snuff probably contains 1-2 g, and the sales figures of portion-bag snuff did not increase substantially until the last 4 or 5 yr. However, as seen from data in Table 6 the most important consumption factor for the development of clinically more severe lesions was "daily hours of snuff use" while "grams of snuff used daily" and "years with regular snuff habit" had comparatively less impact. For instance, a consumption increase from 1 to 10 g a day

corresponds to an increase of the relative risk from 1.06 to 1.70 and the use of snuff for 1 yr compared with 10 yr corresponds to relative risks of 1.04 and 1.53, respectively. As for number of years with regular snuff habit this calculation should be evaluated with some caution since the P-value is comparatively high. The use of stepwise logistic regression to evaluate the relative importance of various exposure data gives somewhat contradictory information compared with previous studies. It should be stressed that this calculation is based on the clinical visible lesion and has no implications for deeper tissue changes. However, by far the most promotive factor for the development of clinically more severe lesions was the package form, with a relative risk for loose versus portion-bag snuff of 3.3 (Table 6). This was elucidated by carefully matching two subjects according to tobacco exposure data (Figs. 3-4).

Alternatively, differences between the severity of the lesions could be explained by the different composition of tobacco used in loose and portion-bag snuff. However, as shown in Table 5, for all three pairs of products compared, the clinical lesions associated with the portion-bag snuff were less severe.

An attempt has also been made to assess the importance of previous smoking and alcohol habits on the observed differences in clinical gradings between users of loose and portion-bag snuff. There is no evidence that either of these factors considerably influenced these differences.

In previous studies, gingival recessions related to the use of smokeless tobacco have been observed. The present study has established clinical criteria making it possible to compare recessions associated with use of snuff in the two different package forms. The present finding of 23.5% gingival recessions registered in users of loose snuff is a somewhat lower figure than previously reported (13-15), but this might be due to criteria differences. However, such differences can probably not explain the remarkably low percentage (2.9%) of gingival recessions among users of portion-bag snuff.

The findings of this study indicate that the use of portion-bag snuff is associated with less severe clinical oral mucosal lesions and a lower frequency of gingival recessions compared with such lesions among users of loose snuff.

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Original articles

Histologic changes associated with the use of loose and portion-bag packed Swedish moist snuff: a comparative study

Andersson G, Axéll T, Larsson, Å. Histologic changes associated with the use of loose and portion-bag packed Swedish moist snuff: a comparative study. *J Oral Pathol Med* 1989; 18: 491-497.

This study was to identify histologic tissue changes in the oral mucosa and to compare them in specimens from users of loose can-packed and portion-bag-packed moist snuff. The material consisted of biopsies from 252 regular snuff users, 184 using exclusively loose and 68 portion-bag snuff. An array of structural changes appearing in different combinations were identified among the 252 specimens. Two major patterns were recognized based on changes in the surface layer. Type 1 was characterized by an increased epithelial thickness with vacuolated cells and frequent chevron type changes. Type 2 showed a variably thickened surface layer with evidence of keratinization. Based on these findings, 14 carefully matched pairs of loose and portion-bag users were analyzed and compared. Loose snuff users showed predominately histologic Type 1 changes while portion-bag users showed more histologic Type 2 or only very discrete changes.

Key words: mouth, disease; oral mucosa, pathology; snuff; tobacco, smokeless.

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Use of smokeless tobacco is a common habit in many countries. However, the products are of many different types (1). In Scandinavia the overwhelming majority of snuff dippers use non-fermented moist snuff. During the last decade a new smokeless tobacco product, portion-bag packed snuff, has been introduced. Clinical changes associated with this type have recently been described (1), but no report of their histomorphology has been published.

Histologic changes seen in "snuff dipper's lesion" have been described in several previous studies (2-12). Common are hyperplasia of the epithelium with large vacuolated cells, some of which have been described as koilocytosis (12) and a chevron type of keratinization (10). In the connective tissue variable degrees of inflammation, amorphous changes, sialadenitis and other degenerative changes in lip sali-

very glands have been observed (5, 7, 8, 11, 12). Slight epithelial dysplasia has been reported but the true frequency is a matter of dispute (4, 5, 7, 8, 12).

This study was to identify snuff-associated histologic tissue changes in the oral mucosa and to compare them in specimens from users of loose can-packed and portion-bag-packed moist snuff.

Material and methods

Material and examination procedure - The material for this study consisted of biopsies from 252 volunteers, all of whom were healthy men, regular snuff users for at least the last three months and with no other tobacco habit. For a detailed description of the recruitment procedure see ANDERSSON & AXÉLL (1). Loose snuff was used by 184 sub-

jects of mean age 36.0 ± 11.6 yr, range 19-80 yr and portion-bag snuff by 68 individuals of mean age 36.9 ± 9.9 years, range 17-66 yr. Users of loose snuff consumed 23.6 ± 12.2 g/day during 10.8 ± 3.8 h and had done so for 13.1 ± 8.2 yr. The corresponding figures for portion-bag users were 11.3 ± 4.9 g/day during 10.3 ± 3.2 h and for 3.1 ± 2.5 yr.

At a first visit, all subjects were examined according to a standardized program, including questions on snuff habits, e.g. package form, brand, duration of habit, daily consumption and specified placement of the quid (one or more sites). A thorough clinical examination was carried out. Changes at the site(s) where the snuff was regularly placed were registered according to a four-grade clinical scale (7).

Among users of loose snuff, 10 (5.4%) Degree 1, 33 (17.9%) Degree

Oral pathology

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Table 1. Age and snuff exposure data of 28 matched subjects.

	Product	
	Loose snuff <i>n</i> = 14	Portion-bag snuff <i>n</i> = 14
Age, yr	34.7 ± 12.1	36.9 ± 10.0
Hours of daily snuff use	11.5 ± 3.4	11.2 ± 3.2
Grams of snuff used daily	17.0 ± 5.5	15.8 ± 4.9
Years with regular snuff habit	10.3 ± 8.0	4.4 ± 2.8

2, 130 (70.7%) Degree 3 and 11 (6%) Degree 4 lesions were registered (1, 7). Among users of portion-bags 13 (19.1%) subjects showed Degree 1, 31 (45.6%) Degree 2 and 24 (35.3%) Degree 3 lesions. No Degree 4 lesion was registered in this group. The higher proportion of clinically less severe changes among users of portion-bags compared with loose snuff users was statistically significant ($P < 0.001$).

Biopsy procedure - From the central part of each changed area a biopsy was taken with a 6 mm punch using local anesthesia as infiltration well separated from the area of biopsy. The specimen was fixed in 10% neutral buffered formalin and embedded in paraffin. Five μ thick sections were stained with hematoxylin-eosin and PAS. Sections were also stained with rhodamine B and examined by fluorescent light (13) to evaluate the degree of keratinization. Sections of all 252 biopsies were examined light microscopically and compared with normal mucosa.

HPV-immunocytochemistry - Biopsies showing koilocytosis-like changes (14) were examined immunocytochemically, for the presence of HPV-antigen. Deparaffinized sections were incubated at room temperature with 0.15% H_2O_2 in methanol for 30 min, for blocking of endogenous peroxidase. Following a short rinsing in phosphate-buffered saline (PBS, 0.1 M pH 7.2), the sections were incubated with normal goat serum for 30 min followed by overnight incubation at 4°C with goat anti-human HPV (Dakopatts) diluted at 1:200 or 1:400. Negative controls included sections in which the primary antibody was omitted. Sections of a viral wart, run in parallel with the other sections, served as positive controls.

For immunostaining, the ABC-technique was used. Sections were incubated with biotinylated anti-goat IgG for 30 min, rinsed with PBS and incubated with the peroxidase-conjugated biotin-avidin complex for 30 min (Vectastain). Following further PBS rinsing, peroxidase activity was localized by incubating with 3,3'-diaminobenzidine \times 4 HCl (DAB 0.6%, Sigma) in 100 ml 0.05 M Tris (pH 7.6) with 0.01% H_2O_2 for 1 h. Counterstaining was done with Mayers hematoxylin.

Matched pairs - In the clinical evaluation of the material the influence of package form and exposure data on the development of clinically more pronounced changes was assessed by means of stepwise logistic regression. Based on this evaluation 14 carefully matched pairs of loose and portion-bag users were selected (Table 1). The matched paired subjects used the same brand of tobacco either loose or wrapped in paper-bags, placed in the same site, and they exhibited closely similar patterns of snuff exposure in terms of hours of daily use and grams. Despite efforts to match for years of regular snuff habit there is a considerable difference between the groups due to the fact that portion-bags have only been available for a comparatively short time. The distribution of clinical degrees of changes among the matched subjects is shown in Table 2.

Based on findings in all 252 biopsies a selected number of histopathologic criteria (see below) were applied when studying the biopsies of the 14 matched pairs in an effort to identify traits specifically related to the package form of snuff.

Results

Histomorphology of the normal mucosa

In the present study, all the biopsies were taken from the midportion of the changed area, corresponding to the inside of the upper lip, close to the vestibular fornix. According to SCHROEDER (15), the epithelium of this part of the oral mucosa differs structurally from that of the buccal and of the alveolar mucosa. The epithelium is composed of small, ovoid, basophilic basal cells, which rather abruptly transform into a voluminous and pale "hydrosium" layer, with large polyhedral cells with a very low dye affinity. These cells seem to have a low density of cytoplasmic filaments and a high density of cytoplasmic ground substance. In routinely

prepared sections (formalin fixation and paraffin embedding), they therefore often exhibit a vacuolated appearance (Fig. 1). In the upper-most stratum distendum¹, they tend to form a more or less sharply flattened but still nucleated surface layer and these cells are variably condensed, forming a thin surface layer with pyknotic nuclei (Fig. 1). The final step of differentiation does not normally lead to a homogeneous surface layer in this part of the oral mucosa.

Histomorphology of the snuff-associated changes

In comparison with corresponding mucosa of a non-snuff user (cf. Fig. 1), an array of structural changes appearing in different combinations were identified among the 252 snuff users. Variable degrees of non-specific chronic inflammation were observed in all cases. The other changes have been defined as described below and these criteria have subsequently been applied when analysing specific subgroups of cases.

Changes of the surface layer - Two major patterns were recognized. In Type 1, the surface layer had an increased thickness and was composed of vacuolated cells with or without visible remnants of nuclei (Fig. 2). This feature was a commonly encountered finding and was always rhodamine B negative. It was not infrequently combined with a "chevron type pattern" (10) of piled-up, more stained cells with more well-preserved nuclei. These chevron type changes ("Christmas trees") tend to occur in those areas of the epithelium which cover the top of the connective tissue papillae (Fig. 2).

Type 2 showed a variably thickened surface layer with an eosinophilic stain and with a variable number of pyknotic

Table 2. Distribution of 28 matched subjects according to clinical grading.

Package form	Clinical degree*			
	1 <i>n</i> = 2	2 <i>n</i> = 9	3 <i>n</i> = 17	Total <i>n</i> = 28
Portion-bag snuff	2	7	5	14
Loose snuff	-	2	12	14
Total	2	9	17	28

* No clinical Degree 4 was encountered within the matched material.



Fig. 1. 45-yr old man. Biopsy taken from normal mucosa not exposed to snuff, corresponding to area inside upper lip, in which snuff-users place their quids. A non-keratinized surface to area inside upper lip, with some artefactual spillo, exhibiting flattened cells with pyknotic nuclei. "Hydrosium" layer is composed of pale swollen cells. Distinct, basal cells form well-stained layer bordering underlying connective tissue. $\times 180$.

nuclei (Fig. 3). A more or less continuous rhodamine B stain was always observed in this layer and/or in spots corresponding to the level of the granular layer.

Combinations of Type 1 and 2 were also identified, either appearing separately from each other or mixed together within the same surface area. Type 1 changes combined with a thin homogeneous structure-less surface zone, staining eosinophilic were also registered (Fig. 2). This zone always stained negative with rhodamine B (Fig. 8).

Atrophy and hyperplasia - This merely refers to the thickness of surface epithelium. In the photographically documented sections (24 \times 36 mm negative size), we have arbitrarily defined the epithelium to be hyperplastic when a given area could not be completely reproduced within the horizontally oriented film rectangle at an original $\times 50$ magnification (cf. Figs. 1-2). Accordingly, and in order to picture completely a hyperplastic epithelium within the given film area, the epithelial surface had to be orientated parallel to the short side of the film. In comparison with the thickness of normal epithelium, "epithelial hyperplasia" would then correspond approximately to a 1.5-2 fold increase of thickness.

Similarly, the epithelium has arbitrarily been defined to be atrophic when a more or less extensive loss of rete pegs

was observed in conjunction with an over-all reduction of the thickness (Fig. 4).

Increased mitotic rate - The rate was recorded as increased, when more than two mitotic figures could be identified within any localized epithelial area at high power (orig $\times 100$), corresponding to a surface area of approximately 0.08 mm² (Fig. 5).

Koilocytosis - Vacuolated epithelial cells are common in changes associated with snuff dipping (Fig. 2) frequently resembling truly koilocytotic cells. Such vacuolated cells are also a characteristic finding in normal epithelium of this part of the oral cavity (Fig. 1). In snuff dipper's lesions, vacuolated cells are often found to be piled up within the epithelium, sometimes extending all the way from the surface down to the basal cell layer. Problems may arise interpreting these cell changes as normal, degenerative or truly koilocytotic.

In snuff changes, vacuolated cells were classified as degenerative, due to the chemical etching of snuff, when appearing in continuity with the surface layers and extending into the deeper epithelial layers (Fig. 6). Accordingly, cells were interpreted as koilocytosis-like (cf. 14) when appearing in small narrow clusters with no obvious connection with otherwise continuous layers of degenerative cells. These presumably koilocytotic cells showed a

vacuolated cytoplasm appearing as a clear rim round a pyknotic nucleus (Fig. 7).

Increased cellular density and basal hyperplasia - In some snuff-induced changes, the degree of vacuolization of epithelial cells may be decreased rather than increased, in contrast with what is commonly observed in such changes. They give the impression of an increased cellular density, partly due also to a better dye-affinity of the cytoplasm of these cells (Fig. 5). An increased density may also be due to a changed nuclear-cytoplasmic ratio (Fig. 5). No distinction has been made between these two patterns but both have been recorded as "increased cellular density". Occasionally, increased density is mainly localized to the basal part of the epithelium, "basilar hyperplasia" (Fig. 8).

Other histologic observations - Other observations have been made, all previously known from other studies of changes associated with snuff dipping, but appearing in the present study with such a very low frequency, that meaningful interpretations were difficult or impossible to make. Among these, eosinophilic leukocytes (7) were observed within the epithelium in a few cases. Similarly, eosinophilic connective tissue amorphous or hyaline changes (2, 7) could be recorded in only about 10 cases. Salivary gland involvement (8) could not be recorded in a meaningful way, due to the highly variable inclusion of gland tissue in the biopsies.

Epithelial changes suggestive of dysplasia, as defined by the criteria of WHO (16), were observed in only a few isolated cases. These findings will be the subject of a separate publication.

HPV-immunocytochemistry

Koilocytosis-like changes were identified in 17 cases. We were however unable to demonstrate convincingly positive HPV-antigen immunoreactivity in any of these biopsies. Further, none of these showed any dysplasia.

Matched pairs

We were able to match 14 pairs of loose and portion-bag snuff users (Table 2). No case of unequivocal dysplasia was recorded. Histomorphologic changes were distributed as shown in Table 3.

All the 28 cases showed some degree of non-specific inflammation, but we



Fig. 2. 35-yr old man with clinical Degree 4 lesion following dipping with loose snuff (14 h daily, 40 g a day, 16 yr with regular habit). Type 1 surface change. Surface layer is heavily thickened due to piled-up vacuolated cells, with remnants of nuclei. Outermost surface zone is homogeneous and eosinophilic but it is rhodamine B negative. Cases exhibiting this type of surface change frequently fulfilled criteria of "hyperplasia". Spikes of cells within vacuolated layer form "chevron pattern". These spikes are clearly related to underlying connective tissue papillae (arrow), in this case slightly inflamed. No dysplasia. $\times 185$.

were unable to detect any clear-cut differences between the two groups of snuff users. Cases with hyperplasia and increased mitotic rate were evenly distributed between the two groups. No Degree 4 lesion came out among the matched cases. When examined pairwise, we found that within each of six matched pairs (1-6), the clinical changes were of an identical grade, five (Nos. 1-5) of these also showing histologically almost identical surface changes. In the remaining eight pairs (Nos. 7-14), the portion-bag users showed clinical changes of a lower degree than their matched loose snuff users. Three of these pairs showed almost identical surface Type 1 changes (Nos. 7-9), but

in the other five (Nos. 10-14), histologic surface changes differed within each of the pairs. Here, four of the loose snuff users showed clinical Degree 3 lesions (Nos. 10-13), three of these having Type 1 surface changes (Nos. 10-12). In contrast, all of the five portion-bag users had Degree 1-2 lesions (Nos. 10-14) and they all exhibited a histologic Type 2 change or a normal-looking epithelium.

These findings indicate that based on comparable snuff habits, loose snuff may cause clinically more pronounced changes (Degree 3) accompanied by histologic Type 1 changes. Portion-bag snuff, associated with less pronounced changes (Degrees 1-2) show more of

histologically Type 2 or only very discrete changes.

Discussion

Snuff, as used here, causes clinical and histomorphologic changes of the mucosa. Among these, epithelial changes have attracted much attention, but detailed correlative clinical-histopathologic studies are still inconclusive.

By comparing two different habits of snuff use, we have been able to identify three major histologic patterns of surface change:

1. A thin eosinophilic surface zone, with opacified cells. We interpret this



Fig. 3. 28-yr old man with clinical Degree 3 lesion following dipping with loose snuff (10 h daily, 17 g a day, 9 yr with regular habit). Type 2 surface change. Surface layer is keratinized (rhodamine B-positive, not illustrated). This is accompanied by slight inflammation and increased density of epithelial cells, exhibiting increased stainability, slightly enlarged nuclei and clearly visible nucleoli (cf Fig. 1) but no dysplasia. $\times 185$.

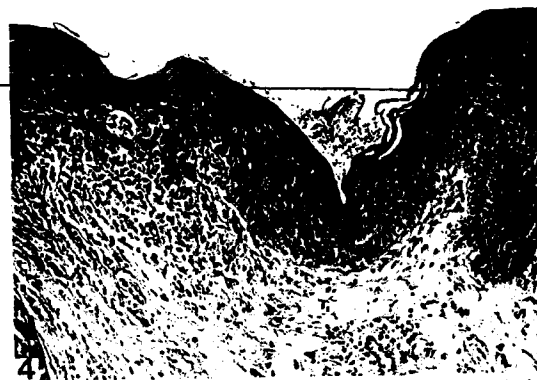


Fig. 4. 23-yr old man with a clinical Degree 2 lesion following dipping with portion-bag packed snuff (13 h daily, 24 g a day, 3 yr with regular habit). This corresponds to atrophy of the epithelium combined with keratinization (Type 2 surface change) and a moderate degree of inflammation. No dysplasia. $\times 180$.

as a coagulative necrosis due to chemical etching by the snuff. It was variably observed in both habit groups. It may be misinterpreted as a keratinized layer but it stains negative with rhodamine B. We think that it may readily desquamate shortly following removal of the snuff quid. Hence, this layer is a variable finding in biopsies and not related to any specific habit of snuff use.

2. Vacuolization or swelling of the surface cells combined with aberrant desquamation causing a "pseudohyperplasia" of the epithelium. This is our Type 1 change which we interpret as indicative of an osmotic imbalance presumably caused by cell membrane alteration resulting in an osmotic absorption of water into the injured cells. This was a common finding in both groups of snuff users. The depth of vacuolated change varied, but was rarely found extending through the whole epithelial thickness. Thus, chemical cell membrane alteration by snuff is mostly restricted to the surface layers. The vacuolated cell surface change was occasionally accompanied by the characteristic "chevron pattern". Histomorphologically, this phenomenon seems to be the result of a persistence of more well-preserved cells, topographically associated with the underlying connective tissue papillae (10). Hence, these cells may be in a more favorable metabolic relationship to the underlying vascularized tissue.

3. The development of a keratinized

surface layer, as evidenced by positive rhodamine B staining (13). This Type 2 change indicates an activation of the keratinization process in this area, which is normally non-keratinized. Such changes were observed in both groups of snuff users.

The crucial question is to what extent any of these three surface changes may be accompanied by serious damage to the underlying epithelial cell layers. Loose snuff tends to be more chemically etching than portion-bag snuff,

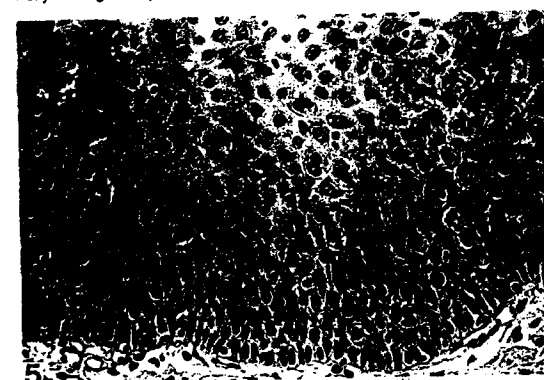


Fig. 5. 18-yr old man with a clinical Degree 2 lesion following dipping with portion-bag packed snuff (10 h daily, 16 g a day, 4.5 yr with regular habit). High magnification of basal-deep spinous cell layer, showing several mitoses (arrows). Spinous layer shows increased density, with enlarged cell nuclei and well stained cytoplasm. No dysplasia. $\times 360$.

i.e. causing more deeply-reaching effects, resulting in piling-up of significant numbers of vacuolated cells. All our cases of "hyperplasia" showed this characteristic change. Impeded desquamation may contribute to this thickening which may perhaps be associated with alterations of keratinocyte membrane lipids. This may be related to added rigidity and decreased fluidity of the cell membranes induced by tobacco components (17).

Occasionally, keratinization was observed. No studies seem to have been published on the cytokeratin pattern in snuff-induced changes and no conclusions can therefore be drawn about the way in which snuff may cause such a change. However, based on the present finding, keratinization per se is not accompanied by epithelial changes other than those seen in cases lacking keratinization.

GREER & POULSON (12) found koilocytotic changes in 26 of 45 snuff users and 6 of these 26 were HPV-positive. In contrast to this, we were unable to demonstrate HPV-antigen in any of the biopsies exhibiting koilocytosis-like changes. Perhaps with other techniques such as DNA hybridization, more information can be gained about any role played by HPV or other viruses in "snuff dipper's lesion".

The present study focused on the comparison between package forms and the 14 pairs were selected accordingly. No case suggestive of dysplasia was found within the matched material.



Fig. 6. 22-yr old man with clinical Degree 3 lesion following dipping with loose snuff (16 h daily, 17 g a day, 8 yr with regular snuff habit). Thick vacuolated surface layer is in direct continuity with vacuolated cells also in deeper spinous layers, the latter having koilocytosis-like appearance, with pyknotic nuclei. $\times 185$.



Fig. 7. 32-yr old man with clinical Degree 4 lesion following dipping with loose snuff (13 h daily, 40 g a day, 11 yr with regular habit). Type 1 surface change, cf. Fig. 2. Localized area of deep spinous/parabasal layer shows koilocytosis-like cells (arrow) not clearly forming a continuum with swollen surface cells, cf. Fig. 6. $\times 185$.

This could possibly be explained by the fact that regular long-term snuff users were not included due to the matching procedure (5). An increased mitotic rate was observed in several cases of both groups and in a few cases accompanied by an increased cellularity. In addition to inflammation these were the only changes found in the deeper tissue layers. Our interpretation is that they represent a reactive response to the surface change caused by the snuff. The damaging potential of snuff to the mucosa is however a controversial subject, especially concerning the incidence rate of precancerous/dysplastic development in snuff lesions (18). In the present study we have not yet completed our analysis of possible irreversible changes, which will require repeated biopsies.

In a previous study, based on a clinical grading system (1), loose snuff was



Fig. 8. 41-yr old man with clinical Degree 3 lesion following dipping with portion-bag packed snuff (7 h daily, 12 g a day, 10 yr with regular habit). Slightly swollen, opacified, rhodamine B-negative surface layer. Increased density is observed in basal parts of epithelium, "basilar hyperplasia". $\times 180$.

Table 3. Matched pairs of snuff users. Histologic changes defined according to text.

Pair No.	Accession No. ¹	Surface layer ^{2,3}	Hyperplasia (H) atrophy (A)	Mitoses	Cell density ⁴	Clinical degree
1	018	1	-	+	+	3
	243	1	-	+	-	3
2	062	1	H	-	-	3
	278	1	-	-	-	3
3	061	1	H	+	-	3
	364	1(2)	H	+	-	3
4	041	1	H	+	-	3
	353	1(2)	-	-	-	3
5	017	1	-	+	-	3
	244	1	A	-	-	3
6	037	2	-	+	+	2
	252	1	H	-	-	2
7	014	1(2)	-	-	-	2
	378	1	H	-	-	3
8	038	1	H	-	-	2
	292	1	-	+	-	3
9	056	1	-	+	+	2
	772	1	-	-	-	3
10	023	2	-	+	-	2
	318	1	H	+	-	3
11	074	2	A	-	-	2
	314	1	-	-	-	3
12	054	2	-	-	-	2
	279	1	-	-	-	3
13	031	N	-	-	-	1
	298	2	H	-	-	3
14	060	N	-	-	-	1
	334	2	-	+	+	2

¹ first case in each pair uses portion-bag snuff, second case loose snuff.

² (2) Type 2 present in addition to predominant presence of Type 1.

³ N = normally-looking mucosa.

⁴ + includes basilar hyperplasia and/or generally increased density.

found to be associated with a higher frequency of clinical Degree 3-4 lesions than portion-bag packed snuff. In the present study, having matched the 14 pairs with respect to amount and time of exposure per day of snuff, we found that clinical Degree 3 lesions were predominantly found among the loose snuff users. These were predominantly associated with histologic Type 1 changes with osmotically swollen cells. Among the portion-bag users, we found a tendency to exhibit Type 2 changes, with evidence of keratinization. Interestingly, all of these type 2 changes were associated with clinical Grade 2 lesions. Taken together, these findings indicate that portion-bag packed snuff results in less pronounced changes to the oral mucosa than loose snuff, but in the present study we have been unable to selectively study the effects of differences in habits. Further

studies are in progress to elucidate this problem.

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Impact of consumption factors on soft tissue changes in Swedish moist snuff users: a histologic study

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Andersson G, Axéll T, Larsson Å: Impact of consumption factors on soft tissue changes in Swedish moist snuff users: a histologic study. *J Oral Pathol Med* 1990; 19: 453-8.

The purpose of this study was to analyze the relative importance for histologic changes of the oral mucosa of such consumption factors as years with regular snuff use, hours and grams of daily snuff use. The material consisted of biopsies of selected cases (two groups) from 252 regular snuff users of whom 184 used exclusively loose and 68 exclusively portion-bag packed snuff. Group 1 comprised 8 pairs of loose snuff users with large differences (14-45 yr) in terms of years with regular habit. Many years of snuff use did not per se seem to result in tissue changes which significantly differed from changes seen in subjects with only a few years of loose snuff use. Group 2 included 5-10 subjects showing the lowest and highest daily consumption of loose or portion-bag packed snuff. Among those with a low daily consumption, portion-bags seem to be related to less pronounced changes than loose snuff. High daily snuff use was associated with relatively somewhat more pronounced epithelial surface changes but histologic differences between the two habit groups were difficult to identify.

Key words: mouth, diseases; oral cavity; snuff; tobacco, smokeless.

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It is well known that snuff causes changes of the oral mucosa, and that the clinical appearance may vary with differences in consumption factors (1-3). Recently it was found by means of step-wise logistic regression calculation that both the form of the snuff (loose versus portion packed) and the placement of the quid (one site versus multiple sites) had a relatively significant impact upon the clinical appearance of the mucosal changes. Number of years with regular snuff habit seemed to have less impact than daily exposure to snuff in terms of hours using snuff daily and grams of snuff used daily (4). In other previous studies (5, 6) these factors have been applied to a simple cumulative snuff exposure index based on the assumption that each of the individual consumption factors has an equally important influence on the development of the oral lesions. This has, however, not been clearly verified.

The purpose of the present study was to analyze the relative importance of consumption factors, such as years with regular snuff use, hours of daily snuff use and grams of snuff used daily on the histologic changes found at the site

Table 1. Age and snuff exposure data of study population

	Loose can snuff n=184	Portion-bag snuff n=68	Total n=252
Mean age, yr	36.0±11.6	36.9±9.9	36.3±11.2
Range, yr	19-80	17-66	17-80
Hours of daily snuff use	10.8±3.8	10.3±3.2	10.6±3.6
Grams of snuff used daily	23.6±12.2	11.3±4.9	20.3±12.0
Years with regular snuff habit	13.1±8.2	3.1±2.5	10.4±8.4

Table 2. Eight matched pairs of loose snuff users according to daily consumption and with large duration differences

Pair No.	Age, yr	Accession No.	Consumption			Clinical degree	Predominant histologic type
			Year	g/day	h/day		
1	51	321	20	12.5	2	3	1
	46	310	3	12.5	4	1	normal
	38	354	20	14.3	6.5	2	2
2	21	399	1	14.3	5	3	2
	50	246	35	12.5	7	2	1
3	25	278	4	12.5	6	3	1
	41	272	17	16.7	10	3	1
4	39	292	3	16.7	9	3	1
	66	239	50	25	10	3	2
5	27	224	5	25	10	3	2
	47	234	25	20	15	3	1
6	20	349	5	20	13	3	1
	60	248	40	25	15	3	1
7	26	364	5	25	13	3	1
	63	306	40	33.3	14	3	2
8	45	266	6	33.3	14	3	1

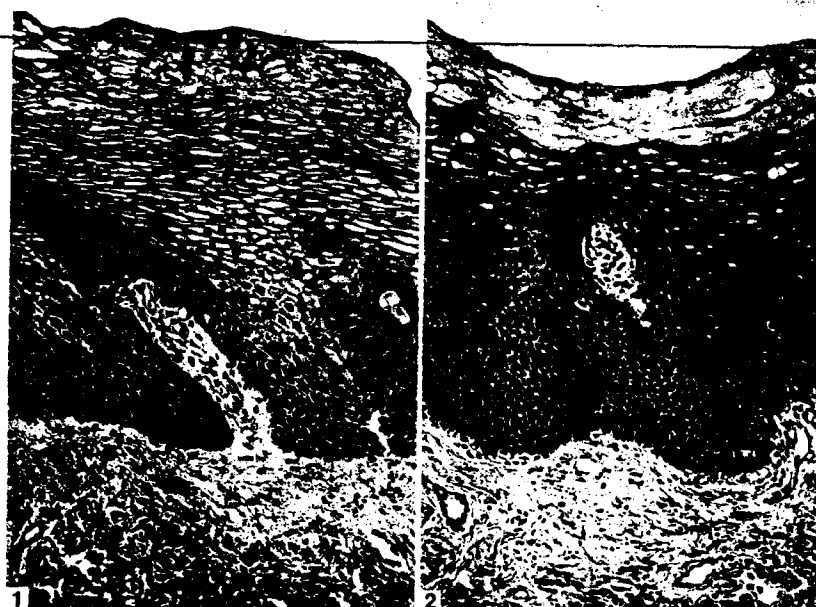


Fig. 1. Matched pair No. 3 (acc. No. 246, Table 2). 50-yr-old man with clinical degree 2 lesion (loose snuff, 7 h daily, 12.5 g a day, 35 yr with regular habit). Type 1 surface changes with vacuolated cells, a thin acrotic, eosinophilic surface zone (arrow) and a slight inflammation in connective tissue. This biopsy showed increased mitotic rate but no dysplasia. Cf. Fig. 2 for comparison with short duration. $\times 200$.

Fig. 2. Matched pair No. 3 (acc. No. 278, Table 2). 25-yr-old man with a clinical degree 3 lesion (loose snuff, 6 h daily, 12.5 g a day, 4 yr with regular habit). Type 1 surface changes with vacuolated cells combined with insignificant connective tissue inflammation. No increased mitotic rate, no dysplasia. Cf. Fig. 1 for comparison with long duration. $\times 200$.

where the snuff quid was regularly placed.

Material and methods

Included in the study were 252 healthy men with a regular snuff habit for at least the last 3 months and with no other current tobacco use. For a detailed description of the recruitment procedure, see ANDERSSON & AXÉLL (4). Age and snuff exposure data are shown in Table 1.

All subjects were called to a dental clinic and asked about type and brand of snuff used, years with snuff usage, daily use in terms of hours and grams and whether they placed the snuff quid at one or more sites. Further questions were asked about general health, medi-

cation, other tobacco habits and alcohol consumption. A careful clinical examination was carried out including registration of snuff dipper's lesions classified according to a four grade clinical scale (7).

From the central part of each changed mucosal area a biopsy was

Table 3. Selected cases with low daily consumption of portion-bag packed and loose snuff

Type	Age, yr	Accession No.	Consumption			Clinical degree
			g/day	h/day	yr	
P-bag	43	009	8	5	1	2
	66	020	4.4	5	7.5	1
	33	025	6.9	5.5	2	1
	40	036	5.3	5	4	2
	48	048	6.9	7	9.0	1
Loose	37	217	7.1	2.5	10	2
	32	274	3.6	1	16	1
	33	339	7.1	3.5	13.5	1
	44	281	6.7	5.0	10.0	3
	33	288	7.1	6.0	18	1

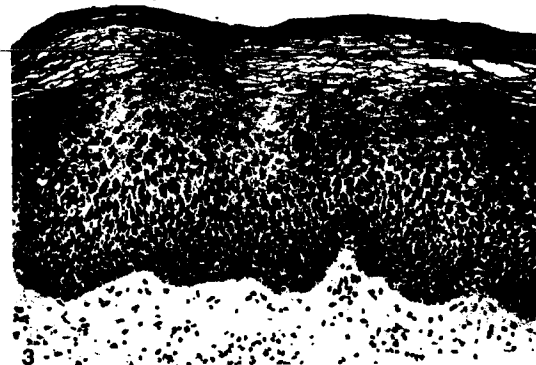


Fig. 3. Low consumer of portion-bag packed snuff (acc. No. 030, Table 3). 40-yr-old man with a clinical degree 2 lesion (5 h daily, 5.3 g a day, 4 yr with regular habit). Close-to-normal surface epithelium. The surface layer is eosinophilic with pyknotic nuclei but with no evidence of keratinization (rhodamine B neg.). A slight degree of inflammation in connective tissue. No increased mitotic rate. Cf. Fig. 4 for comparison with low consumption of loose snuff. $\times 190$.

taken with a 6 mm punch instrument. The tissue was fixed in 10% neutral buffered formalin and embedded in paraffin. Five micron thick sections were stained with hematoxylin-eosin and PAS. Sections were also stained with rhodamine B and examined by fluorescent light (8) to evaluate the degree of keratinization.

Sections of all 252 biopsies were examined light microscopically. An array of structural changes appearing in varying combinations were identified among which, "type 1" or "type 2" surface changes were characteristic findings (9). In type 1, the surface layer is variably thickened, combined with vacuolated cells and often showing "chevron-type patterns". This feature is commonly encountered in snuff dipper's lesions (10) but occasionally type 2 changes appear. They are characterised by a variable degree of keratinization, as evidenced by an eosinophilic and a more or less continuous rhodamine B stain (8).

Two main groups both including only subjects placing the quid at one site, were selected for the present study.

This selection was made by one of the authors (GA) and the microscopic examinations by another (AL). The histologic analysis was carried out without any information available about the subject's tobacco consumption patterns.

Group 1 - These patients were selected from the 184 users of loose snuff in

order to evaluate the relative importance of duration of snuff habit (number of years). Based on close to equal daily consumption (hours/day, grams/day) and large differences in terms of years with regular habit (range differences 14-45 yr) we were able to identify 8 pairs according to Table 2. No portion-bag users were included because of lack of cases fulfilling our criteria of long duration.

Group 2 - These patients were selected from the total material of 252 subjects with the purpose of evaluating the relative impact of daily consumption factors (hours/day, grams/day). Based on the findings obtained in Group 1,

Table 4. Selected cases with high daily consumption of portion-bag packed and loose snuff

Type	Age, yr	Accession No.	Consumption			Clinical degree
			g/day	h/day	yr	
P-bag	42	017	24	17	6	3
	44	023	16	14	1.5	2
	34	061	24	13	5	3
	37	015	16	13	5	2
	31	063	16	13	1	2
	44	066	24	13	1	3
	39	070	16	13	1	2
Loose	24	286	33.3	16	9	3
	41	340	66.7	16	21	3
	34	343	50	16	17.5	3
	57	264	25	17	30	4
	38	249	30	15	16	3
	60	248	30	15	12	3
	25	316	30	15	12	3
	39	384	66.7	15	24	3

these patients were selected irrespective of years with snuff habit, according to the following. From the loose and portion-bag users, respectively, the 5-10 subjects showing the lowest and highest daily consumption were selected. The consumption data of these four categories are summarized in Tables 3 and 4.

Results

Many vs. few years of loose snuff use (Group 1)

Data of the eight pairs are summarized in Table 2. In three of these pairs (Nos. 1-3), all the six patients had a daily snuff consumption of no more than 7 h, the corresponding figure being 9-15 h for the remaining five pairs (Nos. 4-8). Clear-cut histologic differences between the individual cases of each separate pair were difficult to identify in the biopsies. The different types of surface changes (type 1, 2) were evenly and seemingly randomly distributed among the subjects, with the type 1 predominating. Four of five cases of epithelial hyperplasia appeared within two of the matched pairs (Nos. 3 and 7) and they all showed the typical type 1 surface change (Figs. 1-2). There was a slight difference in mitotic rate between subjects in some of the matched pairs, with five (acc. Nos. 321, 354, 246, 234, 248) of seven such cases (also acc. Nos. 292 and 364) being long-time users. Notably case No. 292, with only a 3 yr history of snuff use showed an increased mitotic rate whereas it matched case No. 272, with a 17 yr history, showed no such change. Neither did cases Nos. 306 and 239, with a 40 and 50 yr history, respectively, show any increased mitotic rate. In

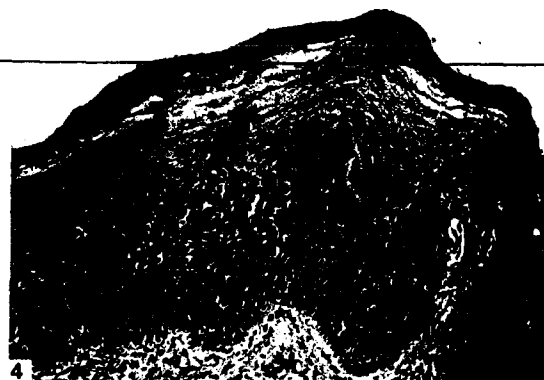


Fig. 4. Low consumer of loose snuff (acc. No. 281, Table 3). 44-yr-old man with a clinical degree 3 lesion (5 h daily, 6.7 g a day, 10 yr with regular habit). Close-to-normal surface epithelium. A thin eosinophilic surface zone lacks evidence of keratinization (rhodamine B neg.). A few inflammatory cells in connective tissue. No increased mitotic rate. Cf. Fig. 3 for comparison with low consumption of portion-bag packed snuff. $\times 190$.

cases Nos. 234 and 354 a slightly increased cellular density was found. No case suggestive of dysplasia (cf. 8) could be demonstrated in any of the 16 cases.

From these findings, we conclude

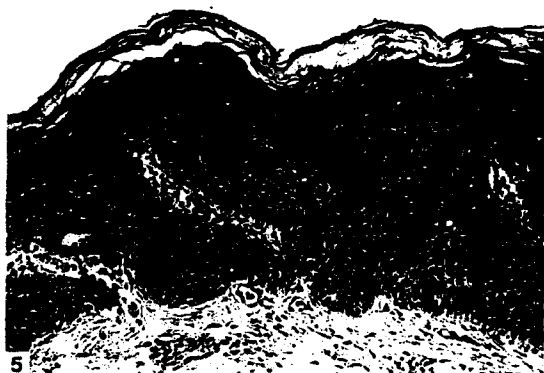


Fig. 5. High consumer of portion-bag packed snuff (acc. No. 066, Table 4). 44-yr-old man with a clinical degree 3 lesion (13 h daily, 24 g a day, 1 yr with regular habit). Type 1 surface change, with moderately thickened vacuolated surface layer accompanied by an increased mitotic rate in the underlying epithelium (not illustrated) but no dysplasia. Discrete inflammation in connective tissue. For comparison Cf. Fig. 3 = low consumption of portion-bag packed snuff and Fig. 6 = high consumption of loose snuff. $\times 190$.

High vs. low daily consumption of loose and portion-bag packed snuff (Group 2)

Low consumption of portion-bag packed snuff—Five cases were identified, using 8 g snuff or less for no more than 7 h a day (Table 3). Three of these five had a close-to-normal surface epithelium (Fig. 3). One had a well-developed type 1 and one had a type 2 surface change, the latter also showing slightly atrophic epithelium. No case with increased mitotic rate was recorded.

Low consumption of loose snuff—Five cases were found using 7.1 g or less for no more than 6 h daily (Table 3). All of these five showed a thin, homogeneous eosinophilic surface zone (Fig. 4). Two of them were combined with a thin type 1 change. No case with increased mitotic rate was recorded.

High consumption of portion-bag packed snuff—Seven cases were identified, using 16 g snuff or more for at least 13 h a day (Table 4). Five of these had a typical type 1 surface change, being prominent in three and less extensive in two (Fig. 5), but none of the five showing hyperplasia (9). Two of the seven cases showed a type 2 change. An increased mitotic rate was found in six cases.

High consumption of loose snuff—Eight cases were identified using 25 g or more of loose snuff for at least 15 h a day (Table 4). One case showed type 2 and seven the typical type 1 surface change, being prominent in five and accompanied by hyperplasia in four (Fig. 6). One of the two cases, showing a less prominent type 1 change, also had areas of type 2. This case (acc. No. 264, Table 4) was the only clinical grade 4 case, and was interpreted as suggestive of dysplasia also showing an increased density and increased mitotic rate (Fig. 7). The latter was also being found in five more of the eight cases.

In summary, in comparison with low consumption, high daily consumption of portion-bag packed or loose snuff results in more pronounced surface epithelial changes. Predominant among these are the appearance of type 1 changes, with swollen cells variably accompanied by a thin necrotic surface zone, and with an increased mitotic rate. Among those with a high consumption histologic differences between the two snuff habit groups were difficult to identify with the exception of a single case using loose snuff. Among those with a low consumption, portion-bag packed snuff tends to cause less changes than



Fig. 6. High consumer of loose snuff (acc. No. 248, Table 4). 60-yr-old man with a clinical degree 3 lesion (15 h daily, 25 g a day, 40 yr of regular habit). Prominent type 1 surface change with swollen cells ("hyperplasia") and evidence of chevron-pattern, also accompanied by an increased mitotic rate (not illustrated) but no dysplasia. A moderate degree of inflammation in connective tissue. For comparison, Cf. Fig. 4 = low consumption of loose snuff and Fig. 5 = high consumption of portion-bag packed snuff. $\times 200$.

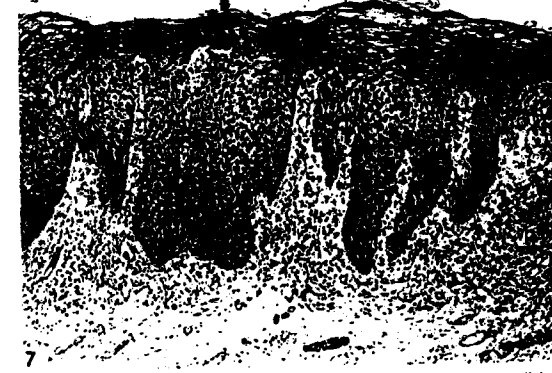


Fig. 7. High consumer of loose snuff (acc. No. 264, Table 4). 57-yr-old man with a clinical degree 4 lesion (17 h daily, 25 g a day, 30 yr with regular habit). Type 1 and 2 surface changes are accompanied by slightly irregular rete pegs. An increased cell density is present in lower part of epithelium, accompanied by evidence of loss of cohesion (arrow) as well as increased mitotic rate (not illustrated) and connective tissue inflammation. These changes were interpreted as suggestive of dysplasia. $\times 190$.

loose snuff. Only one case suggestive of dysplasia was recorded in the subgroups.

Discussion

Supporting previous findings in the clinical part of this study, it was difficult to clearly identify definite histomorphologic differences in biopsies taken from subjects with a long history (17–50 yr) compared with a short history (1–6 yr) of loose snuff use. Within this group, with the two subjects of each of the eight pairs matched with respect to daily exposure (h/day and g/day) of loose snuff, there was a slight tendency among those with a very long history to show an increased mitotic rate. However exceptions to this were also seen, indicating that long duration may not be a decisive factor for the development of this particular change.

In order to further analyze the impact of daily consumption upon tissue changes, cases were selected based on low (less than 6–7 h/day) and high (more than 13–17 h/day) consumption of each type of snuff. The differences in tissue reactions between users of portion-bag and loose snuff which we identified in the low consumption groups, were no longer observed between the high consumers, with the exception of one single case using loose snuff and also exhibiting the only clinical degree 4 lesion of this selected material. The present findings clearly showed that tissue changes (evidence of surface etching with development of type 1 change; mitotic rate) were more pronounced among subjects exposed to snuff for many hours daily in contrast to a few hours daily.

In a recent clinical study (4), it was suggested that years with regular snuff habit as compared to daily consumption had a rather low influence on the development of changes in the oral mucosa associated with snuff use. Thus, a simple cumulative index, as used in a few previous studies (5, 6), may not truly reflect these changes. This conclusion was further supported by the present histopathologic study, based on the same material as the clinical study referred to above. The daily but intermittent use of snuff causes a mixed tissue reaction of injury and repair. Hence, the tissue response observed at any given time must be a result of the combined effects of hours and grams of snuff used daily and of the time permitted for tissue repair. Also, the tissue response should some-

how be modified by the duration, i.e. the number of years with regular snuff habit.

We conclude that the tissue response following a certain snuff habit is rather consistent and predictable, being predominated by surface etching. Isolated cases will however always appear showing a slightly aberrant tissue response such as our acc. No. 264, a heavy consumer of loose snuff, showing the only clinical degree 4 lesion of the present study (Fig. 7). It is tempting to believe that such aberrations may be related to an individually variable capacity of the mucosa to repair and recover from the snuff-induced changes. Such a capacity seems to be little influenced by many years of regular snuff habit. Rather, our present findings indicate that a decisive factor may be the daily consumption. The histology of possible irreparable changes among snuff users has however not been very well characterized, due to lack of experimental data. Such studies

may require repeated biopsies and are in progress as part of the current project.

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Reversibility of snuff dipper's lesion in Swedish moist snuff users: a clinical and histologic follow-up study

Larsson Å, Axéll T, Andersson G: Reversibility of snuff dipper's lesion in Swedish moist snuff users: a clinical and histologic follow-up study. *J Oral Pathol Med* 1991; 20: 000-000.

The purpose of this study was to assess possible reversibility of oral mucosal changes, associated with the use of Swedish moist snuff, after change of habit. Biopsies from a total material of 252 regular snuff users, 184 using loose snuff and 68 using portion-bag packed snuff, were screened microscopically to identify histopathologic epithelial changes in addition to, or differing from, those generally seen as a result of snuff use. The main basis for selection of these variables were the criteria of epithelial dysplasia as defined by WHO and the eight histologic features, which have been reported to be the most important discriminators to separate cases with leukoplakia that subsequently developed carcinoma, from those that did not. Twenty-nine subjects, 3 showing Degree 2 lesions, 21 Degree 3 lesions and 5 Degree 4 lesions, all of them loose snuff users were identified. All the 29 users were re-examined clinically and histologically after 3-6 months. The rebiopsy was always secured from the same mucosal area as the original biopsy. At follow-up, 20 subjects had either stopped their snuff habit or changed to portion-bags and changed placement of the quid. All of them showed a healthy mucosa at the previous biopsy site and normal tissue in the histologic examination of the rebiopsies. Seven subjects had changed to portion-bags and variably reduced their daily exposure to snuff. At follow-up they presented with less pronounced clinical changes and the rebiopsies showed evidence of reduced epithelial changes. One major conclusion from this study is that tissue changes, clinically as well as histologically, are reversible following cessation of snuff habit. This statement is also valid for such parameters used to define dysplasia which may occasionally occur in snuff dipper's lesion.

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It is frequently stated that the habit of snuff dipping is harmful to the oral tissues. The question of whether mucosal changes caused by snuff show histologic features of dysplasia or carcinoma is, however, a matter of dispute. This is because it is not known to what extent the parameters usually used to define dysplasia (1, 2) occur in tissue alterations observed in snuff dippers, often referred to as snuff dipper's lesion. Furthermore, if parameters characteristic of dysplasia are found in snuff dipper's lesion, it is not known whether or not the presence of these parameters is predictive of carcinoma.

This dispute may be due to lack of experimental data or to ill-defined specifications of type of tobacco (3). A complicating factor may be a lack of consensus regarding the criteria, as well as the

use of the term dysplasia. For example, KAUGAR *et al.* (4) equated dysplasia with "pre-malignant lesion" using microscopic features accepted by WHO (1) as the determining criteria. They noted that 90.9% of their selected cases demonstrated "epithelial dysplasia" at the site of tobacco placement. No follow-up data were, however, provided. Conversely, SMITH *et al.* (5) examining 15,000 snuff users, found that none of 157 biopsied patients with clinically detected mucosal change showed signs of dysplasia or carcinoma after 5 years of follow-up. Of these 157 cases, 128 were followed for another 4.5 yr, but no patient showed signs of dysplasia or malignancy (6). SMITH *et al.* (5) concluded that there was no reliable data pointing to the ability of unburned tobacco to cause oral neoplasia. They further

stated that without well-documented clinical studies or experimental evidence, many writers and clinicians seem to accept an association between mouth cancer and long-term snuff use. It seems that this 20-yr-old statement is still valid, at least with respect to the types of snuff consumed by the Swedish community (about 4500 tons a year by about 800,000 individuals). However, it is well recognized that mucosal changes regularly occur in snuff dippers, (7, 8), but our knowledge about the biologic nature or behavior of such changes is limited.

The purpose of the present study was to assess the possible reversibility of lesions after change of habit in cases originally showing some, but not all, of the parameters of dysplasia, by examining follow-up biopsies.

Material and methods

The original material consisted of biopsies from 252 healthy men with regular use of non-fermented Swedish moist snuff for at least the last 3 months and with no other current tobacco use; 184 used loose snuff and 68 used portion-bags. For a detailed description of the material see ANDERSSON & AXELL (7). The histopathology of those biopsies has been generally characterized in a previous publication (8). In short, the biopsies were found to present a fairly consistent pattern of changes. Among the loose snuff users, predominantly "type 1" surface changes were found, featuring non-keratinized swollen surface epithelial cells with or without a chevron-type pattern as well as an accompanying eosinophilic surface layer indicative of the chemical etching effect of the snuff. These changes were sometimes combined with epithelial hyperplasia and an increased mitotic rate, and invariably with connective tissue inflammation. Similar changes could be detected among the users of portion-

bag packed snuff. "Type 2" changes, characterized by a keratinized (rhodamine B positive) surface layer, were more often recorded among portion-bag users than among loose snuff users.

In the present study all the 184+68 original biopsies were screened microscopically with the purpose of identifying histopathologic epithelial changes in addition to or differing from those generally seen as a result of snuff use as described above. Twenty-nine subjects, all of them loose snuff users, were identified. The distribution of clinical grading was: 3 cases showed Degree 2 lesions, 21 cases Degree 3 lesions and 5 cases Degree 4 lesions. For detailed information on consumption and clinical grading see Table 1. All the 29 snuff users were re-examined clinically after 3-6 months, following efforts to have them stop, or at least change their habit. The biopsy for microscopic re-evaluation was taken at least 6 months after change of habit and was always secured from the same mucosal area as the original biopsy. As a group for comparison, 5 loose-snuff users were selected based

on a daily consumption of at least 25 g consumed during 12 h or more. For detailed description see Table 1. Sections were stained with H-E, PAS, rhodamine B or Congo Red.

In the microscopic re-examination of the 184+68 original biopsies, a group of epithelial changes were identified in some of the biopsies, including various combinations of increased mitotic rate, increased cell density (including "basilar hyperplasia", 8) and loss of cell cohesion. Some other slight changes, including the criteria of epithelial dysplasia as defined by WHO (1), were also observed. In order to determine their significance, an effort was made to assess subjectively the absence or presence of a number of different variables, which have been deemed important in previous, snuff-related studies. The main basis of selection of these variables was the eight histologic features, which KRAMER (9) reported to be the most important discriminators to separate cases with leukoplakia who subsequently developed carcinomas, from those that did not. The parameters KRAMER suggested were used with slight modifications and additions, as follows:

- 1 Abnormal mitoses in the stratum basale and/or spinosum, and/or an overall increased mitotic rate, as defined previously (8).
 - 2 Disturbance of polarity of basal cells, in which the long axis of the cells is no longer clearly perpendicular to the basement membrane.
 - 3 Nuclear hyperchromatism, with few or groups of cells, mostly basal, staining more intensely than neighboring cells.
 - 4 Russell bodies in the lamina propria.
 - 5 Enlarged nucleoli.
 - 6 Pleomorphism, with variations in cell and nuclear shape.
 - 7 Intraepithelial keratinization.
 - 8 Loss of epithelial intercellular cohesion, with or without accompanying inflammation.
 - 9 Basal cell hyperplasia with an increased thickness of the basal cell layer resulting in "increased density" (8).
- In addition to these variables, eosinophil infiltration was recorded, as a possible potent mediator of tissue damage (10) and thereby a possible contributor to loss of intercellular cohesion (p. 8 above). The degree of hyaline change in the lamina propria was also recorded to seek a possible relationship with any of the other variables included in the study. Based on recent reports of possible disturbances of the mitotic process by

Table 1. Age and snuff exposure data of 29 follow-up cases

Group	Accession No.	Age	Consumption			Clinical Degree
			h/day	g/day	Yr	
1	264	57	17	25	30	4
	255	29	10	33.3	10	3
	212	23	5	9.1	8	4
	369	27	11	6.2	6	3
	268	28	14	28.6	10	3
	204	70	14	12.5	8	4
2	331	21	8	33.3	3	3
	209	34	12	40	17.5	4
	216	25	6	14.3	5	3
	284	49	15	16.7	14	3
	287	25	14	25	12.5	3
	343	34	16	50	17.5	3
	345	26	7	16.7	11	3
	365	21	10	25	3	3
	388	40	10	12.5	12.5	3
	386	32	13	40	11	4
	227	21	10	28.6	8	3
	366	47	12	12.5	25	3
	248	60	15	25	40	3
	291	41	13	33.3	11	3
	314	54	15	25	5	3
	363	42	7	8.3	10	2
	377	30	15	50	15	3
	384	39	15	66.7	24	3
	374	33	8.5	20	3.5	3
	245	22	3	12.5	3	3
	315	29	11	16.7	11	3
3	238	23	10	20	5	2
	313	48	16	5	25	2
Cases for comparison	226	39	12	25	20	3
	338	51	14	40	7	3
	351	29	14	25	13	3
	229	47	16	25	29	3
	289	58	12	25	12.5	3

Table 2. 29 follow-up cases. Histologic changes defined according to text

	Accession No.	Increased mitotic rate	Loss of cohesion	Increased cellular density	Eosinophil infiltration
Group 1	264	+	+	+	+
	255	+	+	+	+
	212	+	+	+	+
	369	+	+	+	+
	268	+	+	+	+
	204	+	+	+	+
Group 2	331	+	+	+	+
	209	+	+	+	+
	216	+	+	+	+
	284	+	+	+	+
	287	+	+	+	+
	343	+	+	+	+
	345	+	+	+	+
	365	+	+	+	+
	388	+	+	+	+
	386	+	+	+	+
	227	+	+	+	+
	366	+	+	+	+
	248	+	+	+	+
	291	+	+	+	+
	314	+	+	+	+
	363	+	+	+	+
	377	+	+	+	+
	384	+	+	+	+
	374	+	+	+	+
	245	+	+	+	+
	315	+	+	+	+
Group 3	238	-	-	+	+
Group 4	313	-	+	+	+
	226	-	-	-	-
	338	-	-	-	-
	351	-	-	-	-
	229	-	-	-	-
	289	-	-	-	-

tobacco treatment (11), binucleate spinous cells were recorded. Biopsies were arbitrarily regarded as positive, if at least two binucleate cells could be found within the confines of any individual rete peg.

Results

All the 184+68 original biopsies showed some degree of inflammation in the connective tissue. A few (*vide infra*) showed hyaline change, which was Con-

go red/amyloid negative. No cases with binucleate cells, abnormal mitoses, Russell bodies, intraepithelial keratinization or prominent nuclear hyperchromatism could be recorded. All biopsies showed some degree of nuclear enlargement, with visible nucleoli. Only occasionally a case with a slight degree of pleomorphism or some loss of basal cell polarity was observed. The histopathologic findings of 29 loose snuff users exhibiting in their original biopsies some degree of epithelial change in addition to what could be generally observed as a result of snuff use (p. 1-9 above), is summarized in Table 2. Among portion-bag snuff users, no cases showing corresponding changes were found. Based on the findings, we arbitrarily subdivided the 29 loose snuff users into four subgroups for re-biopsy analysis, as follows.

Group 1 - Seven cases, which all showed a combination of an increased mitotic rate, increased cell density and evidence of loss of cohesion. Six of these showed eosinophils and four had hyaline changes in the lamina propria. Four of them (acc Nos. 264, 255, 212, 369) had given up the snuff habit at follow-up and showed a healthy mucosa at the previous biopsy site and normal tissue in the histologic examination of the re-biopsies (Figs. 1-3). Among these four was the only case (acc No. 264, Fig. 2) exhibiting a clinical Degree 4 lesion in our previous study of the impact of consumption factors (12). Among the remaining three cases, one subject (acc No. 268) had exactly the same habit at the follow-up, again presenting with a clinical Degree 3 lesion and with an un-

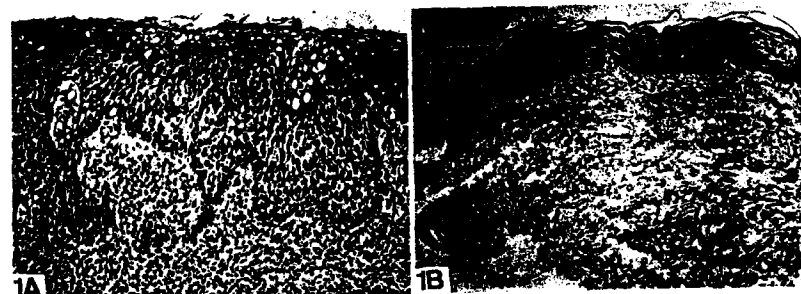


Fig. 1 A, B. (Acc No 212). 22 yr old man with clinical Degree 4 lesion following use of loose snuff (5h daily, 9.1 g a day, 8 yr with regular habit). Original biopsy (A) showing non-keratinized epithelium with irregular rete pegs, combined with inflamed connective tissue. Epithelial surface cells are swollen and necrotic. Loss of cellular cohesion is evident. This biopsy also showed increased mitotic rate, localized areas of basal hyperplasia and eosinophilic granulocytosis. Following stop of snuff habit, rebiopsy taken 1 yr later (6 months after he stopped to use snuff) showed normal epithelium, with only a few inflammatory cells in the connective tissue (B). A: $\times 140$. B: $\times 70$.

changed histologic picture. Two individuals (acc Nos. 204 and 331) had changed to portion-bags, had reduced their daily consumption (204: 6h/d, 5g/d; 331: sporadic use) and now placed the quid at several different sites. At the follow-up they both had clinical Degree 1 lesions at the original biopsy site. Histologically, inflammation and minor epithelial surface changes were recorded but increased mitotic rate, cell density and cohesion loss was no longer seen.

Group 2 – Twenty cases with an increased mitotic rate and an increased cell density but without loss of cohesion. None of these had eosinophils or hyaline change in the lamina propria. Eleven of these (acc Nos. 209, 216, 284, 287, 343, 345, 365, 388, 386, 227, 366) had stopped the snuff habit at least three months before the follow-up examination. Clinical-

ly, as well as histologically, they showed a normal mucosa at the site of rebiopsy (Fig. 4). Four subjects (acc nos. 248, 291, 314, 363) had changed to portion-bags and reduced the amount of grams used daily but they exposed the mucosa for the same number of hours as before. They had also changed the placement of the snuff. At the follow-up, one of these (acc No. 248) had a clinical Degree 2 lesion while the other three had Degree 1 lesions at the site where the tobacco was held. However, all four had a normal looking mucosa at the previous biopsy site, which was supported by the histologic analysis. Among the remaining five cases, four had changed their habit to portion-bag packed snuff (acc Nos. 377, 384, 374, 245). They had the same daily exposure time to snuff but had reduced the amount consumed to about one-half.

All four showed clinical Degree 2 lesions at the time of rebiopsy. Histologically, in contrast to the original biopsies, the rebiopsies showed no increased mitotic rate or increased cell density. One individual (acc No. 315) continued to use loose snuff, but he had reduced the daily exposure slightly to 11 g/d during 9 h and placed the snuff at two different sites. At the follow-up he still presented with a clinical Degree 3 lesion although somewhat less marked (thinner). Histologically, in addition to type 1 surface changes, a few mitoses were recorded but no increased cell density.

Group 3 – One case (acc No. 238) had an increased cell density but without increased mitotic rate or loss of cohesion. This case showed eosinophils and hyaline changes. At the follow-up he had not modified his snuff habit in any

way and presented with a clinical degree 2 lesion similar to the original examination. The histologic picture was also the same at both occasions.

Group 4 – One case (acc No. 313) had loss of cohesion and increased cell density but without an increased mitotic rate (Fig. 5A). The original biopsy also showed eosinophils. He stopped his snuff habit and at the follow-up 6 months later, the previously recorded Degree 2 lesion had disappeared and the mucosa looked normal. The rebiopsy also showed normal tissue when examined microscopically (Fig. 5B).

Cases for comparison

Three of the five cases showed type 1 surface change, the other two type 2. Three cases (acc Nos. 226, 338, 351) showed normal mucosa, clinically and histologically, at follow-up. They had either discontinued their snuff habit (acc No. 338) or changed to portion-bags and changed the placement of the snuff quid (acc Nos. 226, 351). The two cases showing persistent changes (acc Nos. 229, 289) had only modified their habits slightly.

Discussion

In the present study, approximately 10% of all the biopsies, 29 cases from a total material of 184 loose snuff and 68 portion-bag packed snuff users, as described previously by ANDERSSON & AXÉLL (7), exhibited epithelial alterations different from or in addition to what was subjectively found to pre-

dominate in such lesions, as previously described (8). The purpose of the present study was to define the possible reversibility after change of habit, of such epithelial alterations. Five "prototype" cases served as rebiopsy controls. For practical purposes, we have been unable to rebiopsy all the remaining cases.

Although the selection of the 29 cases, who exclusively used loose snuff, was based on an initial subjective analysis of the 184 + 68 biopsies, there was still an empirical foundation for the selection in the sense that it was based on the use of histologic criteria, usually used to assess the presence and degree of dysplasia (1, 2). Following rebiopsies of these 29 cases, we then reapplied a complete set of nine different histological parameters, which closely correspond to those found by KRAMER (9) to be important predictors of subsequent malignancies in cases with oral leukoplakias. Our present findings suggest that snuff dipper's lesion may occasionally show some, but not all, of such histologic changes. These changes include various combinations of an increased mitotic rate, an increased basal cell density and a loss of cell cohesion. With the exception of enlarged nucleoli, which was a more or less constant finding in all the 184 + 68 biopsies, none of the remaining five parameters could in fact be found in the present material.

Irrespective of the histologic changes originally observed in the biopsies, all those cases in the present study who discontinued completely their snuff habit, were found to exhibit a normal mucosa in their rebiopsies. Thus, we

found reversibility in 20 of the 29 cases. Of the remaining nine cases, seven showed evidence of reduced epithelial changes, associated in all cases with a variably reduced use of the snuff. One major conclusion from this study must therefore be, that the changes we have found to be present in the 29 cases, including also in the five additional cases for comparison, are reversible following cessation of the snuff habit. Among the seven cases showing the most extensive combination of KRAMER's (9) parameters, (our group 1) four returned to normal in the re-biopsy following cessation of snuff use whereas two of the remaining three cases showed reduced changes following reduced daily snuff use and change of product. Since these changes correspond to some of the parameters used to define dysplasia, an additional conclusion must be that the changes do not correspond to "dysplasia" if dysplastic lesions are thought to be irreversible.

There are several previous studies, reporting no (5, 6, 13) or mild to moderate dysplasia in snuff dipper's lesion (4, 14, 15, 16, 17, 18). The majority of these studies lack histologic illustrations. Interestingly, we have found that the few illustrated cases do not show changes which markedly differ from any of those we have observed in the present 29 rebiopsied cases.

An interesting observation in the present study was that seven of the nine cases showing loss of intercellular cohesion also exhibited eosinophilic infiltration, and that seven of the eight cases showing eosinophils also had evidence

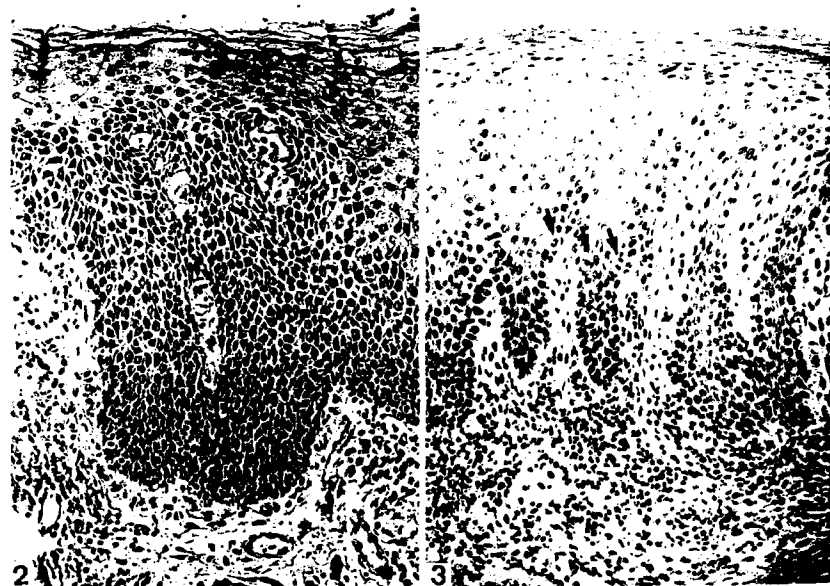


Fig. 2. (Acc No. 264). 57-yr-old man with clinical Degree 4 lesion following use of loose snuff (17 h daily, 25 g a day, 30 yr with regular habit). Original biopsy, showing epithelial hyperplasia with swollen or necrotic surface cells and some connective tissue inflammation. Slight degree of basilar hyperplasia is seen as well as loss of cohesion. This biopsy also showed increased mitotic rate and eosinophilic granulocytes. Following stop of snuff habit, rebiopsy taken 18 months later (6 months after he stopped to use snuff) showed normal tissue, similar to Fig. 1B. $\times 200$. Fig. 3. (Acc No. 369). 27-yr-old man with clinical Degree 3 lesion following use of loose snuff (11 h daily, 6.2 g a day, 6 yr with regular habit). Original biopsy, showing epithelium with slightly drop-shaped rete pegs, basilar hyperplasia and necrotic surface zone. Clearly increased mitotic rate was recorded (arrows) as well as evidence of loss of cohesion with eosinophilic granulocytes also within epithelium. Connective tissue is heavily inflamed. Following stop of snuff habit, rebiopsy taken 6 months later (6 months after he stopped to use snuff) showed normal tissue, similar to Fig. 1B. $\times 200$.

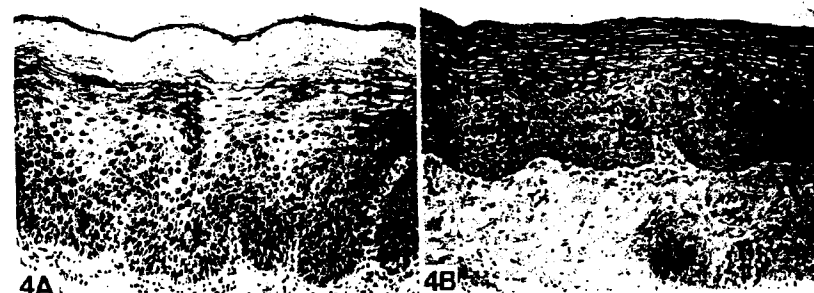


Fig. 4A, B. (Acc No. 209). 34-yr-old man with clinical Degree 4 lesion following use of loose snuff (12 h daily, 40 g a day, 17.5 yr with regular habit). Original biopsy (A) showing epithelium with rounded, slightly drop-shaped rete pegs and thick surface layer of vacuolated cells ("Type 1 change"), accompanied by necrotic surface zone and also some connective tissue inflammation. Increased mitotic rate was recorded (arrows) as well as some basilar hyperplasia but no obvious loss of cell cohesion and no eosinophilic granulocytes. Following stop of snuff habit, rebiopsy taken 2 yr later (18 months after he stopped to use snuff, but he did not allow rebiopsy until 2 yr after the original one) showed normal tissue (B). $\times 140$.

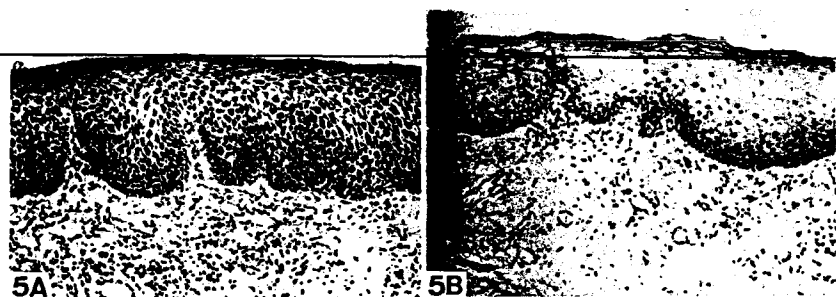


Fig. 5 A, B. (Acc No. 313). 48-yr-old man with clinical Degree 2 lesion following use of loose snuff (16 h daily, 5 g a day, 25 yr with regular habit). Original biopsy (A), showing somewhat atrophic epithelium with flattened surface cells, evidence of loss of cellular cohesion and of basilar hyperplasia. Eosinophilic granulocytes were also found. Isolated mitoses were recorded but mitotic rate was not found to be increased. Following stop of snuff habit, rebiopsy taken 6 months later (6 months after he stopped to use snuff) showed normal tissue, with only few inflammatory cells (B), $\times 140$.

of loss of cohesion. Further, none of the 20 cases of our Group 2, with no evidence of loss of cohesion, showed any eosinophils. Eosinophils have been previously reported in snuff dipper's lesions (13) but possible functional connection with loss of cell cohesion has not been emphasized. The occasional infiltration of human tumors by eosinophils has been noted and discussed for nearly 100 yr (19) but their prognostic significance has been questioned (20). The findings by SILBERSTEIN *et al.* (21) suggest that eosinophil infiltration and possible cytotoxic function in tumors may be indirectly mediated via IL-2. We suggest therefore that eosinophil infiltration in snuff dipper's lesion is a secondary phenomenon, as a consequence of the non-specific inflammation invariably found in these lesions, and that the concomitant loss of cell cohesion may be a result of eosinophil cytotoxic functions.

A better understanding of the biological nature of the histologic changes occurring in snuff dipper's lesion is highly desirable. If there are snuff dipper's lesions that are non-reversible, it is obvious that they must look different from what we have reported in the 29 selected cases, with clearly reversible changes. Furthermore, it follows from a definition of dysplasia as "a disturbance in the maturation of the cellular layers of the stratified squamous epithelium" (2, 5, 22) that the disturbed maturation must occur from the basal cell layers and upwards, since this is the direction of differentiation in oral squamous epi-

thelium. By the same necessity, the direct effects caused by placing snuff on the mucosal surface must take place from the surface layers and downwards. From this simplistic view it may be argued, that morphologic evidence of a disturbed maturation, i.e. "dysplasia", must be mixed together in a snuff dipper's lesion with the morphologic changes caused by the direct chemical, etching effects of the snuff. Consequently, the crucial question is to what extent changes corresponding to disturbed maturation can be histologically identified among the direct changes inevitably caused by the snuff.

Based also on observations in the present study, we are inclined to believe that dysplasia has not been present in any of the 29 cases. This is further supported by the fact that in spite of a large number of oral biopsies per year submitted from all over Sweden, we have never recorded a carcinoma developing in a patient with a pre-existing snuff dipper's lesion. The changes recorded in this study are reversible and we, therefore, interpret them as reactive. They represent a well-defined set of changes following defined habits of using defined types of snuff. To avoid oversimplified statements, evaluation of future studies in any given population will decisively depend upon whether tissue changes have been correlated with type of snuff, e.g. dry or moist, pH-values and package form and to what extent the degree of reversibility correlates to detailed histologic changes.

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Clinical classification of Swedish snuff dipper's lesions supported by histology

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From a total material of 184 Swedish users of loose packed moist snuff and 68 users of portion-bag packed moist snuff, cases were selected from subgroups based on a four-point clinical grading scale. The selected material for the study comprised 70 cases (ten from each clinical grade group, no Degree 4 lesion was found among portion-bag users). Features recognized in biopsies from these cases together with findings in previous studies correlated well with the use of a four-point scale for the grading of clinical changes, especially in the context of discriminating lesions for which special efforts should be undertaken to make the patient stop or change the snuff dipping habit and for selecting patients in whom regular clinical follow-up including a biopsy should be carried out. In this article is also discussed the labeling of the clinical oral mucosal changes seen at the site where a quid of snuff is regularly placed. The conceptual use of "snuff dipper's lesions" is recommended instead of e.g. snuff-induced leukoplakia.

Key words: leukoplakia; mouth disease; oral mucosa; pathology; snuff; tobacco; smokeless.

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The habitual use of oral snuff, snuff dipping, is a habit where a pinch of powdered or particulate tobacco is placed in contact with the oral mucosa. In Sweden, by far the most frequent site of placing a quid of snuff is the vestibular area inside the upper lip and almost exclusively non-fermented moist snuff is used (1).

Almost invariably, a clinically visible change can be found on the site of the oral mucosa where the snuff is regularly placed (2). This change has been called snuff dipper's lesion (2-3, 4), snuff dipper's keratosis (5), snuff-induced oral leukoplakia (6), and snuff-induced lesion (7-8, 9). In the present article the label snuff dipper's lesion will be used.

Subdivision of a diagnosis into subgroups may be useful for many reasons. It may be used to study various aspects of a lesion and its different appearances and it may also form the basis for recommendations on clinical routines. A four-point scale for subgrouping snuff dipper's lesions, suggested by AXELL (2), was used in the present study. This scale is based on clinical criteria including wrinkling, thickening and color changes of the oral mucosa.

The four-point scale has previously been used in several studies on snuff related lesions (1, 4, 7, 10, 11). A modified classification has been used by

GREER & POULSON (12) and GREER *et al.* (13) who reduced the four-point scale to a three-point scale, since in their studies of teenagers, adults, and geriatric patients they were able to classify all lesions into one of Degrees 1-3. HOLMSTRUP & PINDBORG (14) have speculated on whether the three-point scale is applicable on a world-wide basis, but they state that this must await future studies. The aim of the present study was to analyse whether the four-point scale is relevant or justified based on its correlation to the histologic appearance in tissue biopsies.

Material and methods

The material for the analysis of the clinical grading comprises 252 men who, at the sampling and recruitment procedures, were all using Swedish moist snuff on a daily regular basis; 184 were using loose snuff and 68 portion-bag snuff. Sampling and examination procedures including the taking of biopsies are detailed in articles by ANDERSSON & AXELL (1) and ANDERSSON *et al.* (10). The distribution of lesions on the four-point scale, as well as consumption data are detailed in Table 3 in (1).

Snuff dipper's lesions were subgrouped according to the mentioned four-point scale (2). Degree 1: a superficial lesion with a color similar to the

surrounding mucosa, and with a slight wrinkling. No obvious mucosal thickening. Degree 2: a superficial, whitish or yellowish lesion with wrinkling. No obvious thickening. Degree 3: a whitish-yellowish-to-brown, wrinkled lesion with intervening furrows of normal mucosal color. Obvious thickening. Degree 4: a marked, white-yellowish-to-brown and heavily wrinkled lesion with intervening, deep and reddened furrows and/or a heavy thickening.

Selected for histologic analysis in the present study were specimens from 10 consecutive subjects from each "clinical lesion group" comprising the following fractions of the total collected material. From loose snuff users were selected all 10 Degree 1 lesions, 10 of 33 Degree 2 lesions, 10 of 130 Degree 3 lesions and 10 of 11 Degree 4 lesions. From portion-bag snuff users were selected 10 of 13 Degree 1 lesions, 10 of 31 Degree 2 lesions and 10 of 24 Degree 3 lesions. No Degree 4 lesion was encountered among portion-bag snuff users. The number of specimens selected for analysis thus totalled 70. Age and consumption data for the subjects from whom the biopsies were secured are shown in Table 1.

Histologic criteria were the same as those used in previous reports based on the total material (10, 11, 15). Among these, two major epithelial surface pat-

Table 1. Age and consumption factors for 10 consecutive subjects of loose and portion-bag snuff users with lesions of Degree 1 through Degree 4.

Product	Clinical grading	Consumption			
		Age Mean \pm SD	h/Day Mean \pm SD	g/Day Mean \pm SD	yr Mean \pm SD
Loose	1	40.2 \pm 8.9	7.1 \pm 4.5	10.6 \pm 6.7	10.5 \pm 5.7
Loose	2	36.0 \pm 8.9	5.9 \pm 1.1	17.1 \pm 9.8	10.4 \pm 6.5
Loose	3	43.2 \pm 18.0	8.9 \pm 3.8	19.8 \pm 9.5	16.6 \pm 3.4
Loose	4	39.6 \pm 14.1	11.8 \pm 4.0	26.5 \pm 13.1	15.8 \pm 7.7
Portion-bag	1	37.9 \pm 15.6	8.1 \pm 4.0	9.8 \pm 3.5	3.5 \pm 3.3
Portion-bag	2	36.7 \pm 5.3	9.2 \pm 3.1	9.9 \pm 4.2	3.0 \pm 2.4
Portion-bag	3	38.0 \pm 7.4	10.7 \pm 3.3	12.6 \pm 5.3	4.2 \pm 2.8

No Degree 4 lesion was recorded, in portion-bag users

terns, Types 1 and 2, were recorded. The most commonly encountered pattern was Type 1, characterized by a variably thickened surface layer, composed of vacuolated cells and often showing "chevron-type patterns". This feature was always rhodamine B negative. Type 2 changes were characterized by a variable degree of keratinization, staining positive with rhodamine B. The surface could also be recorded as a thin homogeneous structureless zone, staining eosinophilic and rhodamine B negative. This necrotic surface zone could be recorded alone or in combination with the Type 1 change.

Results

The histologic findings related to the clinical grading of the lesions are shown in Table 2 and in Figs. 1-8.

Changes of the surface layer - These changes were subtle in Degree 1 lesions and became increasingly pronounced in Degrees 2-4. A necrotic rhodamine B negative surface zone was a common finding either alone or in combination with Type 1 changes. Type 2 changes were most frequent in lesions of clinical Degrees 1 and 2.

Other histologic observations - Atrophy, hyperplasia, mitoses and basal cell hyperplasia were increasingly more often recorded in lesions with a higher clinical degree. However, three cases of hyperplasia in combination with increased mitotic rate were identified in portion-bag users with Degree 1 lesions, also comprising the three lesions with Type 1 surface changes indicated in Table 2. These three portion-bag users had the highest daily exposure to snuff within this group. In those specimens where increased basal cellularity was recorded, an increased mitotic rate was also seen. A combination of atrophy and hyperplasia was recorded in two cases of loose snuff users showing clinical Degree 4 lesions, comprising also the two cases which showed a necrotic surface zone, but no typical Type 1 or Type 2 changes. In the Degree 4 lesions, three specimens showed loss of cohesion. One of these cases also showed a non-specific ulcer.

Various degrees of non-specific chronic inflammation was observed in all cases. Hyalinization in the connective tissue close to the epithelium was found in a few lesions, predominantly of Degree 4.

The observations in both habit groups can be summarized as follows. In Degree 1 lesions of portion-bag users only subtle histologic alterations were seen (Fig. 1), with an occasional Type 1 or Type 2 surface change. Almost all of the portion-bag users exhibiting clinical Degree 2 lesions showed Type 1 (Fig. 2, most common) or Type 2 changes. In addition, 50% of these cases demonstrated an increased mitotic rate. All except one of the portion-bag users presenting with a clinical Degree 3 lesion showed histologic Type 1 changes, often with a necrotic surface zone. The majority of these cases also showed an increased mitotic rate and a few an increased cellular density (Fig. 3). Almost all loose snuff users exhibiting clinical Degree 1 lesions showed evidence of Type 1 epithelial changes, often combined with a necrotic surface zone (Fig. 4). The pattern of tissue changes among the loose snuff users exhibiting a clinical Degree 2 or 3 lesion (Figs. 4, 5) was very much in accordance with that observed of the corresponding lesions of portion-bag users. Clinical Degree 4 lesions, recorded only among loose snuff users, invariably showed more extensive histologic changes, including epithelial atrophy, a high mitotic rate, occasional loss of cohesion and increased cellular density (Figs. 6-8).

Discussion

In the present study, a relatively consistent pattern of tissue changes was recorded, indicating that a relationship frequently exists between the presently used clinical grading of the lesions and the histologic changes. In clinical Degree 1 lesions, there was a slight inflammation, but only subtle, if any, epithelial changes. In Degree 2 lesions, the epithelium showed a slightly increased thick-

ness of the surface layer with or without an accompanying surface zone of necrosis. A considerably thickened surface layer of Type 1 (10) characterized Degree 3 lesions. In Degree 4 lesions, a

marked thickening of the surface layer may be combined with areas of atrophy and of heavy inflammation. The mitotic rate increased with increasing clinical grading. This might be a physiologic

expression of an increased demand for epithelial repair. Increased basal epithelial cell density and hyalinization of the connective tissue were most common in clinical Degree 4 lesions. Loss of cohe-

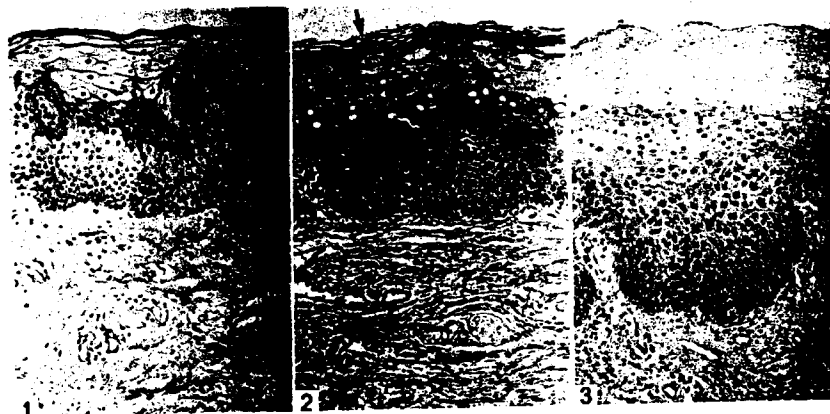


Fig. 1. 48-yr-old man with clinical Degree 1 lesion following use of portion-bag snuff (7 h daily, 8 g a day, 2 yr with regular habit). Normal looking surface epithelium (cf 10) with vacuolated cells typical of this area with only few inflammatory cells. $\times 140$. Fig. 2. 28-yr-old man looking surface epithelium following use of portion-bag snuff (10 h daily, 12 g a day, 1 yr with regular habit). Typical Type 1 histologic change with thickened surface layer of swollen cells, in this case also accompanied by necrotic surface zone (arrow). This case also showed increased mitotic rate (cf arrow head). $\times 140$. Fig. 3. 41-yr-old man with clinical Degree 3 lesion following use of portion-bag snuff (10 h daily, 12 g a day, 10 yr with regular habit). Widely thickened and swollen surface layer (Type 1, cf Fig. 2) and slightly increased basal cell layer is seen. Increased rate of mitoses was also recorded (arrow-heads). $\times 140$.

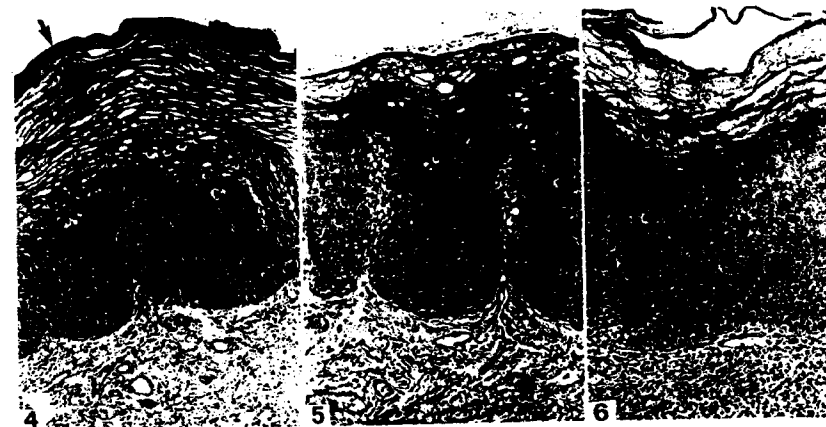


Fig. 4. 48-yr-old man with clinical Degree 1 lesion following use of loose-snuff (13 h daily, 7 g a day, 2 yr with regular habit). Slight change is observed, with thin necrotic surface zone (arrow), small degree of cellular swelling and also some inflammation. $\times 140$. Fig. 5. 49-yr-old man with clinical Degree 2 lesion following use of loose snuff (8 h daily, 7 g a day, 10 yr with regular habit). Thin necrotic surface zone (cf Fig. 4) is accompanied by thickened layer of swollen cells (Type 1 change) and also some inflammation. $\times 140$. Fig. 6. 30-yr-old man with clinical Degree 3 lesion following use of loose snuff (10 h daily, 33.3 g a day, 15 yr with regular snuff habit). Type 1 epithelial change, with thick surface layer of swollen cells, and also some inflammation. This case showed increased mitotic rate. $\times 140$.

Table 2. Histologic features in specimens from 10 consecutive subjects of loose and portion-bag snuff users with lesions of Degrees 1, 2, 3 and 4.

Product	Clinical degree	Surface changes					Atrophy	Hyperplasia	Increased mitotic rate	Increased cellular density	Loss of cohesion
		Normal	Only necrotic zone	Type 1	Type 2						
Loose	1	1	-	8 (6)*	1	-	-	-	-	-	-
Loose	2	0	2	5 (3)	3	3	1	1	1	1	-
Loose	3	0	2	6 (3)	2	2	6	4	1	-	-
Loose	4	0	2	7 (5)	1	5	5	7	5	3	-
Portion-bag	1	1	5	3 (1)	1	-	3	3	-	-	-
Portion-bag	2	1	3	4 (3)	3	1	1	5	1	-	-
Portion-bag	3	0	1	9 (7)	0	3	3	8	2	-	-

No Degree 4 lesion was recorded in portion-bag users.

* () = number of biopsies showing necrotic surface zone adjacent to type 1 change

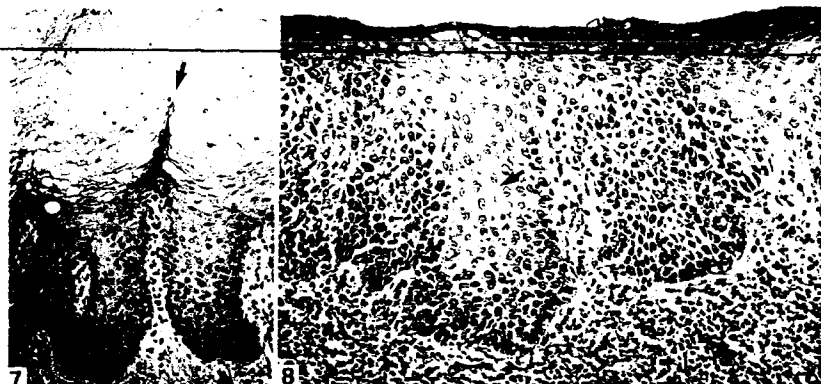


Fig. 7. 35-yr-old man with clinical Degree 4 lesion following use of loose snuff (14 h daily, 40 g a day, 16 yr with regular habit). Heavily swollen surface layer with the typical "chevron pattern" (arrow) and slight inflammation. This case also showed increased mitotic rate and localized areas of epithelial atrophy. $\times 130$. Fig. 8. 70-yr-old man with clinical Degree 4 lesion following use of loose snuff (14 h daily, 13 g a day, 8 yr with regular habit). Slightly atrophic epithelium with thin necrotic surface zone and with evidence of loss of cellular cohesion (arrow) combined with heavy inflammation. This case also showed increased mitotic rate, increased cell density and eosinophilic infiltration (This was reported by LARSSON *et al.* (15) as one of the rebiopsied cases with changed habit, resulting in reduced changes at follow-up). $\times 180$.

sion was only recorded in Degree 4 lesions. These findings indicate that more extensive tissue changes could be expected to be present in clinical Degree 4 lesions compared with findings in Degrees 1-3. These more pronounced changes may, thus, appear in a small fraction of snuff dipper's lesions. The character of these changes has been reported and discussed in detail in a follow-up study on clinical and histologic changes associated with snuff use (15).

It should be emphasized that there is no clear cut difference between each of the clinical degrees, either clinically or histologically and, thus, an overlap between the degrees is logical and sometimes occurs. However, subdivision of snuff dipper's lesion is highly justified. Without subdivision, evaluations and analyses presented in several studies on snuff dipper's lesion would not have been possible. Further, the use of a four-point scale for grading clinical changes obviously gives the opportunity of selecting those snuff dipping patients for whom special efforts should be made to make them stop or change their habit, and also to select those patients for which a regular clinical follow-up program, including a biopsy, should be recommended.

The question whether the grading of snuff dipper's lesion should comprise a three- or four-point scale has been raised (13,14). From the present

findings it may be concluded that the four different clinical degrees employed to register snuff dipper's lesions generally correspond to a fairly consistent set of tissue changes.

In previous articles it has been pointed out that there is a clinical, as well as histologic, difference between the changes encountered among loose and portion-bag snuff users. In this study, histologic differences were observed especially in clinical Degree 1 lesions. With increasing degree, the changes recorded among portion-bag users tend to resemble the histologic pattern of loose snuff users. This is in accordance with previous observations in the analysis of the total material from which the present material was retrieved (10). However, the most important difference in tissue specimens from users of the two package forms of snuff is that, some of the more pronounced histologic parameters e.g. loss of cohesion, were not recorded among portion-bag users. Also no clinical Degree 4 lesion was encountered in this group.

In clinical work and research, diagnostic labels could be helpful tools provided that they are carefully described with clear, easily understandable criteria and also that they are generally accepted. The use of varying diagnostic labels for the same lesion or condition, as for snuff induced oral mucosal changes, is therefore unfortunate. According to a

report from an international seminar on tobacco-related oral mucosal lesions, whitish lesions associated with, and thought to be due to, the use of tobacco should be classified as precancerous lesions of tobacco-associated leukoplakia (16). However, as pointed out by AXÉLL (2, 17) snuff dipper's lesion should not be considered as a form of leukoplakia, but rather as a separate entity like e.g. smoker's palate, because it is not always whitish. Moreover, according to epidemiologic data from Sweden (18) precancerous potential of Swedish moist snuff has not been established.

It is also most likely that whatever substance is regularly placed at a specific site in the oral mucosa some change may appear. Depending on the contents of the "quid" this change may show varying clinical and/or histologic alterations. Such a "quid lesion" should preferably be recorded as a separate entity and not included among leukoplakias, *inter alia* for purposes of follow-up on the development of that specific "quid lesion".

Based on the findings in this study, we suggest that oral mucosal changes associated with the use of snuff should be labelled snuff dipper's lesion and be graded on a four-point scale.

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